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BULLETIN

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CONTENTS.

CONTENTS.

CONTENTS.

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ENTOMOLOGICAL RESEARCH.

STUDIES ON THE TOXICITY OF INSECTICIDE FILMS.*
I. — PRELIMINARY   INVESTIGATIONS   ON   CONCENTRATION-TIME-MORTALITY   RELATION.

By S. PRADHAN.

Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts.

(Plate I.)

CONTENTS.

Introduction.

The importance of the residual effects of insecticidal sprays and dusts has been realised in varying degrees by most of the serious workers in the field of insecticides and fungicides, but Potter (1938) appears to have been the first to give primary importance to it. He proved that it was desirable to spray warehouses not necessarily with the object of hitting the insect directly during treatment but mainly to deposit a protective film on exposed surfaces, so that the moths emerging or flying out of crevices subsequent to spraying might continue to get fatal doses of insecticide on settling on that film. This conception has been definitely consolidated (Potter, 1942, Tattersfield & Potter, 1943, and Parkin & Green, 1943) into what is described as the " film technique ", as distinct from " direct spraying ", for biological evaluation of insecticide toxicity. When these investigators developed this idea they were working with pyrethrum which is one of the less stable insecticides, but the advent of the highly stable DDT immensely enhanced its economic importance. Also, as some of the results presented in the following pages will show, the study of certain insecticides in the film form may be found to be suitable when investigating certain fundamental problems.

Review of Literature.

A perusal of the literature shows that the use of protective films of insecticide by Potter (1938) in the control of insects was opportune, particularly in view of the discovery of the insecticidal value of DDT, the outstanding feature of which is its use in the form of persistent residual films. Since this technique was first experimentally tested by Tattersfield and Potter (1943) and Parkin and Green (1943), its popularity has increased. Dickinson (1944) published a short note on the " technique for studying the residual value of organic insecticides ", which consisted of spraying bean seedlings in pots with a known amount of insecticide per unit area, and infecting them with five adult mites per leaf at various intervals afterwards. Only primary leaves were used and the mites were confined to a definite area by means of a sticky barrier.

In the following year Parkin and Green (1945) published the first critical observation on the toxic property of DDT films, reporting that under certain circumstances toxicity was increased after flies had been once enclosed over it. Morrison (1945) tested the residual actions of DDT and related compounds by using rectangles of filter paper which were immersed in alcohol or acetone solutions, removed while wet, dried and inserted as lining in shell vials in which *Drosophila* adults were kept enclosed for specified periods. Lepage and others (1945) published a technique in which a wooden frame ($9 \times 9 \times 1\cdot 5$ cm.) slotted to receive glass plates or squares of cardboard, treated with insecticides, were used for timed contact of houseflies. Lindquist and others (1945) used the residues of DDT and pyrethrum on sprayed cages for studying the effect of temperature on toxicity. Felber (1945) studied the film formation of certain oil emulsions by spreading in various ways in thin layers on different surfaces, specially glass plates, and observing the successive stages in the process of drying, ultimately leading to various patterns of more or less continuous dry film which could be photographed.

Parkin and Hewlett (1946) studied films of pyrethrum and DDT in heavy oil (shell oil P 31) sprayed on rough deal, sacking, brick, wallboard, cement, cement sand, etc., and using *Tribolium castaneum* adults as test insects, arranged these materials in the order of the toxicities of the films formed on them. Westgate and Bolton (1946) studied insecticide " films " prepared by incorporating DDT and several other insecticides in paints and making thin films of them. Laug (1946) determined DDT in various tissues by putting ether extracts of those tissues in Erlenmayer flasks and after allowing the ether to evaporate, enclosing 100 flies in

the flask containing the residue. Lindquist and others (1945, 1946) published further experiments on the effect of temperature on knock-down and kill of flies, bed-bugs and mosquitos exposed to DDT residues. Sweetman (1945) studied " residual toxicity of DDT " by dusting DDT in petri dishes or on paper towels and storing them for different periods before testing.

During 1947 also, several papers appeared on the study of insecticide films. Kennedy (1947) described the excitant and repellent effects on mosquitos of sublethal contacts with DDT-treated surfaces. He described the behaviour of mosquitos with special relation to their reaction to light. For preparing " the DDT-treated surface ", a standard filter paper was dipped in acetone solution of DDT, drained in saturated acetone vapour, dried thoroughly and weighed before use ". Busvine and Barnes (1947) published their " observations on the mortality among insects exposed to dry insecticidal films ". For preparing them " filter papers were impregnated with solutions of the insecticide calculated to give standard deposits per unit area ". " For impregnation a paper was balanced on pin points and the liquid added spirally from a 1 cc. hypodermic syringe. The paper was fanned to accelerate the first stages of drying . . . and further drying was allowed to continue for a few hours before exposure to insects". Parkin and Green (1947) while working on DDT residual films have described the persistence and toxicity of deposits from kerosene solutions on wallboard. Each square of wallboard was sprayed on one side with insecticide by means of a scent sprayer fitted with a small glass liquid reservoir and worked by compressed air. After spraying, the squares were stored at least four days before use in a room maintained at constant temperature and humidity. By this time the " odour of the solvent was no longer detectable ". Specially reared *Musca domestica* adults were used as test insects. Ten flies were enclosed over each square under an inverted petri dish. In addition to persistence, other factors affecting film formation were considered by them. Webb (1947) has described " a spraying apparatus and testing chamber for investigating the residual action of insecticidal deposits ". Several factors governing the weight of deposit were investigated. The spraying tower was designed to coat panels of 20 cm. square with an even deposit of insecticidal material of known weight. These panels can be built into a cubical testing chamber. MacInnes (1947) has investigated the effects of DDT-treated surfaces on mosquitos and besides films on wood, he described " a film of DDT crystals floating on a water surface ". The crystals " remained floating for 24 hours, even after extensive stirring ".

Besides the above-mentioned laboratory studies there are a number of papers reporting large-scale control of insects by means of the residual effects of DDT (Gahan & Lindquist, 1945, Lindquist *et al.*, 1945, 1946, Bradley & Fritz, 1946, Ribbands, 1947).

Thus a number of papers have appeared during the period in which the present investigation has been in progress. These researches have not, however, necessitated any essential alteration either in technique or the conclusions of this or the second part of these studies to be published later. The time does not appear to be yet ripe for discussing the comparative merits of the different details of the techniques followed by the different investigators.

TECHNIQUE.

The actual details of the technique and material varied from experiment to experiment according to the point under investigation, but as certain operations, material and equipment form common features of more than one experiment, they are described here to avoid repetition.

The general technique involved three major operations : (*a*) preparation of uniform and replicable films, (*b*) keeping the test insects in continuous contact with the film during the desired period and (*c*) assessment of toxic effect.

(a) Film Preparation.

In preparing films no significant deviation was made from the method of Tattersfield and Potter (1943) except that the insects were confined on the film after they had been allowed to dry overnight at constant temperature and not " directly after spraying ". For the majority of the experiments replicable films of the same strength and toxicity were prepared by spraying, in a Potter tower, 5 cc. of solution, emulsion or suspension (as the case may be) of the insecticide on each filter paper (Whatman No. 544) of 9 cm. diameter. Films of different strengths were made by altering the concentration of the insecticide in the liquid to be sprayed but the volume of the liquid and the area of the surface were kept constant. The filter papers were placed under the Potter tower in petri dishes and after spraying they were left in the same dishes to dry overnight in a constant temperature (C.T.) room or cabinet at 80°F. In several experiments the filter papers were substituted by bolting silk of which equal circles of about 9 cm. diameter were cut out.

(b) Keeping Test Insects in Contact with the Films.

The method employed for keeping the insects in contact with the film depended both on the nature of the surface on which the film is made and on the behaviour of the test insect and also on the nature of the insecticide. The following devices have been used :—

(i) *Filter funnels.* These were used for confining *Tribolium castaneum* over films of DDT in the first experiment as they were supposed to be the best for this purpose at the time (Pl. I, fig. *a*).

(ii) *Truncated glass cones cut from filter funnels.* The filter funnels as such were found to be unsuitable when γ-BHC (γ-benzene hexachloride) was used, because they allowed too little ventilation for the escape of fumes. Hence the narrow portion of the funnel was cut away leaving a cone portion used with a diameter ranging from 7 to 4·5 cm. (Pl. I, fig. *b*). These cones were later used even with DDT films because they were found much more convenient in use than funnels. They can, for example, be put on the film and the insects dropped in later through the opening at the top of the cone. Also, if need be, the insects can be inspected without removing the cones.

(iii) *Glass rings cut from wide glass tube.* In some experiments short cylinders (Pl. I, fig. *c*) of about a centimetre or less height cut from glass tube of about 5 cm. diameter were used in place of glass cones described above. These are not so good as the glass cones but they are easier to cut, especially if very little height is needed.

(iv) *Filter paper cones.* The foregoing devices were quite successful with *Tribolium castaneum*, an insect which is incapable of crawling up the sides of the glass enclosures, but they are useless with other insects that can do so and thus remain away from the film. Hence, while experimenting with DDT and *Plutella maculipennis* it was decided to prepare films on two filter papers for each batch of insects and then to convert one filter paper into a cone with film side innermost (Pl. I, fig. *d* — note it is also fitted with an exhaust tube). The insects were enclosed over the film within these cones which were kept pressed down by means of galvanised iron rings of appropriate size.

(v) *Cones of perforated filter paper.* While working with *Plutella* and γ-BHC, the filter paper cones caused trouble on account of lack of ventilation. Attempts were made to overcome this difficulty by perforating a large number of fine holes in the filter paper before spraying and making the cones (Pl. I, fig. *e*). It was necessary for this purpose to have a perforating die so that all filter papers might have the same number of holes arranged in the same corresponding positions. One such perforating die was improvised and perforated filter paper cones worked fairly well

but it proved to be too weak for perforating large numbers of filter papers and a stronger die was not easily procurable.

(vi) *Bolting silk cones.* The perforated filter paper cones were replaced by cones made in the same fashion from sprayed circles of bolting silk (P. I, fig. *f*). These cones are rather flimsy as compared with filter paper cones, but they worked fairly well. The rim of these cones had to be kept pressed on the flat film by means of glass rings which were themselves weighted by iron rings. In these cases the bottom film also had to be made on the same bolting silk so that the nature of surface and film might be similar on all sides.

(c) **Assessment of Effect.**

All the insects (usually 15 per batch) from one film were placed together on a specially constructed warm (C.T.) plate and inspected individually. They were assigned to one of the following five categories : (1) Normal (N)—those in which no sign of abnormality could be noticed. (2) Slightly Affected (S)—those in which the effect was noticeable but which could move about or make some progress. (3) Badly Affected (B)—those which could *not* leave the place where they were but showed vigorous movement of the various parts of the body. (4) Moribund (M)—those which appeared to be dead but on prodding could show some sign of life by feeble movements of some part of the body. (5) Dead (D)—those which showed no sign of life under a magnifying lens despite prodding.

The criteria of these categories are on the whole the same as those of Tattersfield and Potter (1943) except that a stricter line of demarcation was made between the slightly affected and badly affected : all those insects which could leave the place where they were put were assigned to the former category, however severely affected they appeared to be. This rigidity was considered necessary because otherwise there were only differences in degree rather than in kind from " Moribund " to " Normal " and some lack of confidence was felt in judging the difference in degree with the same accuracy each time. Also at times it remained doubtful by mere prodding whether an insect was dead. This doubt often confronted one in the case of *T. castaneum* which has the habit of feigning death, although in case of feigned death the legs and antennae are kept tightly pressed against the body. In all such doubtful cases the insect was put in some irritating fluid like acetone when even very feeble power of movement exhibited itself and those feigning death were at once detected.*

MATERIAL.

The following three species of test insects were used. The details of rearing under approximately standard conditions have been given by Potter (1941), but the following brief notes are given here.

Tribolium castaneum, Hbst.

Only adults were used. A continuous stock of these insects, reared in glass jars on wholemeal flour, has been maintained at constant temperature, for several years in the Department. In each experiment the adults from the same culture or from two cultures started on the same date were used.

Plutella maculipennis, Curt.

All the experiments on this species were carried out with fourth-instar larvae as far as possible of the same size. These insects were reared on potted cabbage plants in the glass house.

Macrosiphoniella sanborni, Gill.

These were reared on chrysanthemum plants in a glass house. In all experiments only wingless adults of approximately the same size were used.

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* They were discarded from further examination after this treatment.

The following insecticides were used :—

(1) DDT :—2, 2, bis (parachlorophenyl) 1, 1, 1, trichlorethane.

(2) γ-BHC :—γ-Benzene hexachloride (γ-Hexachlorocyclohexane).

CONCENTRATION-TIME-MORTALITY RELATIONS.

This study is based on experiments designed to give preliminary information necessary for planning subsequent investigations. Insects confined over films of different strengths, and control insects, were examined at suitable intervals until all died (or pupated in the case of larvae). This procedure automatically combined the three factors : (a) concentration, (b) time, and (c) mortality. The same batches of insects were examined again and again during the course of the same experiment. These numerous examinations may possibly affect results, but it is likely to do so to the same extent in all cases and there are many practical advantages in the procedure. It should, however, be pointed out that the observations after different intervals of time are not independent since the same batches are examined throughout the series of tests.

Concentration-Time-Mortality Relation between DDT and *T. castaneum*.

For this study batches of *Tribolium castaneum* adults were confined over films of DDT and the same batches were examined 17 times at suitable intervals within a period of 36 days.

These experimental details were essentially similar in most of the tests carried out and are given here as an illustration.

29/iii/46. 2·5 gm. of cyclohexylamine dodecyl sulphate (C.H.D.S.) was dissolved in pure benzene and made up to 500 cc. and kept as solvent stock (S) of benzene with 0·5 per cent. C.H.D.S.

S_1–10 gms. of DDT was dissolved in a little quantity of S and made up to 100 cc. with S giving a content of 10 per cent. sol. of DDT.

S_2–1 cc. of S_1 diluted to 100 cc. with S giving a content of 0·1 per cent. DDT.

1/iv/46. The following emulsions were made up :—

> Sc–10 cc. S+90 cc. water (spray medium).
> 1–0·0005% =0·5 cc. S_2+9·5 cc. S+90 cc. water.
> 2–0·001% =1 cc. S_2+9 cc. S+90 cc. water.
> 3–0·005% =5 cc. S_2+5 cc. S+90 cc. water.
> 4–0·01% =10 cc. S_2+90 cc. water.
> 5–0·05% =0·5 cc. S_1+9·5 cc. S+90 cc. water.
> 6–0·1% =1 cc. S_1+9 cc. S+90 cc. water.
> 7–0·5% =5 cc. S_1+5 cc. S+90 cc. water.
> 8–1·0% =10 cc. S_1+90 cc. water.
> C–Unsprayed control.

Three petri dishes of 9 cm. diameter with one filter paper in each were sprayed with each concentration (5 cc. of emulsion at 18 cm. pressure). The dishes were kept after spraying in the C.T. room at 80°F. to dry overnight.

2/iv/46. Fifteen adults of *Tribolium castaneum* were confined under glass funnels of equal diameter on each filter paper, without removing the filter paper from the dish in which it had been sprayed. Three replications were made in each case. After enclosing the insects the dishes were returned to the C.T. room.

3/iv/46 to 8/v/46. Seventeen inspections were made at suitable intervals, the dishes being removed for examination from the C.T. room to the adjoining laboratory. Each batch of insects was examined on the warm C.T. plate and each insect inspected individually and assigned to one of the five categories (p. 5). After examination the dead insects were discarded and the rest were returned to the same film and replaced in the C.T. room.

Although during each observation the insects were separated into five categories it was thought advisable finally to analyse the data on dead insects only, since the insects of other categories were gradually passing into that category as time went on. Table I shows the percentage mortality on the films of different strengths at the times

TABLE I.

Percentage mortality of *T. castaneum* adults on DDT films of different strengths.

Column		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Contact period in days		1	3	4	6	8	10	13	15	17	21	23	25	27	29	31	34	36
Log. contact period		0·0	0·4771	0·6021	0·7782	0·9031	1·00	1·1135	1·1761	1·2304	1·3220	1·3617	1·3974	1·4314	1·4624	1·4914	1·5315	1·5563
Conc. gm./100 cc. sprayed	Log. conc.																	
1·00	0·0000	0	19	38	100	100	100	100	100	100	100	100	100	100	100	100	100	100
0·5	$\bar{1}$·6990	0	16	27	93	100	100	100	100	100	100	100	100	100	100	100	100	100
0·1	$\bar{1}$·0000	0	7	7	60	100	100	100	100	100	100	100	100	100	100	100	100	100
0·05	$\bar{2}$·6990	0	2	2	26	91	100	100	100	100	100	100	100	100	100	100	100	100
0·01	$\bar{2}$·0000	0	0	0	0	9	22	34	55	79	95	100	100	100	100	100	100	100
0·005	$\bar{3}$·6990	0	0	0	0	0	0	12	14	27	54	63	67	76	83	97	100	100
0·001	$\bar{3}$·0000	0	5	7	7	7	7	7	9	9	20	22	31	49	72	87	97	100
0·0005	$\bar{4}$·6990	0	2	2	2	2	9	9	12	12	17	29	33	53	68	80	93	95
Sc	—	0	0	0	2	2	5	5	5	5	11	16	34	52	68	89	93	100
C	—	0	0	0	0	0	0	5	7	12	31	38	43	62	74	86	98	100

of different inspections. The strength of the film is denoted by the concentration of DDT in the emulsion used for making the film. In calculating percentage mortality at the time of any inspection, the total number of insects dead up to date has been used. Thus in Table I the figure 100 per cent. shown in row 1, column IV also includes 38 of column III which in turn includes 19 of the previous column.
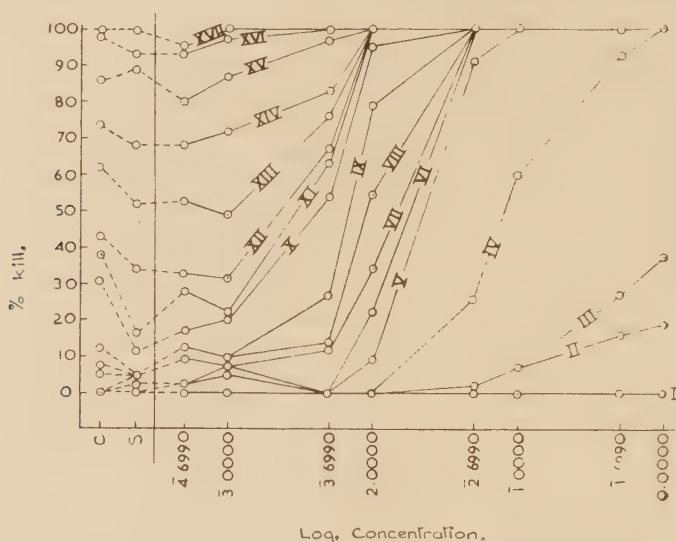
Fig. 1.—Per cent. mortality-log. concentration relation after various periods of contact between *T. castaneum* adults and DDT films.

Concentration-mortality relation. The relation between concentration and mortality is depicted in fig. 1. The concentrations have been plotted on a logarithmic scale as the range of 1 per cent. to 0·0005 per cent. cannot be conveniently represented on an arithmetic scale but this logically excludes the expression of the figures obtained in the controls ; these are given for purposes of comparison, on the left side against the point labelled C and S. An adjustment for percentage in the mortalities in the control can be made by the use of the so-called Abbott formula*.

Each curve in these figures shows the observation made at one time. Thus in fig. 1 there are 17 curves, I–XVII, for 17 sets of observations and it is interesting to notice how the shape of these curves changes with time. There is more or less a gradual change from the horizontal straight line shape of curve I when all insects at all concentrations are alive, to a second horizontal straight line when all insects at all concentrations are dead. The so-called sigmoid curve which is converted into a straight line by the recently developed probit technique comes in between these two extremes. Thus the possibility of getting a good straight line representing mortality probit-log. concentration relation depends on which of these numerous curves we happen to record in our observations. In other words, a good probit line depends on a correct judgment of time for the particular range of concentration and for the particular set of circumstances.

Time-mortality relation. The relation between time and mortality is represented in fig. 2. It will be seen from the figure that it is more or less of the same type as that between concentration and mortality. This similarity, however, cannot hold to

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*The results in this case hardly warrant the publication of a separate figure. Owing to shortage of space, tables have been abbreviated and figures eliminated. They are given in full in the original thesis deposited with the London University.

the extreme limit because there cannot be any lower concentration where the lower horizontal straight line will always remain so, as even the controls must die sooner or later. Similarly there cannot be a concentration sufficiently high for death to be instantaneous and a straight horizontal line showing cent per cent. death at all times is out of the question. The plotting of time on a logarithmic scale does not make any unexpected or essential difference in the shape of these curves. Their shape in this case depends on concentration.

Time in days on logarithmic scale
(log time)
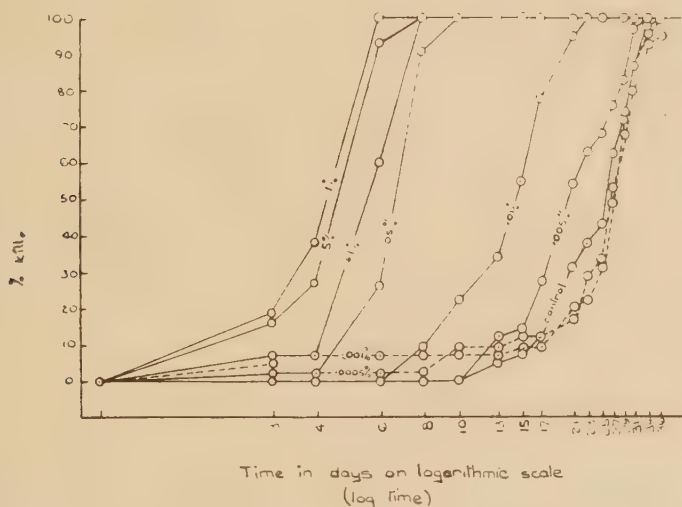
Fig. 2.—Per cent. mortality-log. time relation when *T. castaneum* adults remain in contact with DDT films of various strengths.

Combined Concentration-Time-Mortality Relation. Although figs. 1 and 2 do also represent the combined effect of time and concentration on mortality, an attempt was made in fig. 3 to express this combined effect by the " probit plane method " (Finney, 1947). In this method the values of percentages of Table I (after adjustment with control) were converted into probits according to Fisher and Yates (1938, Table IX). The probit values were plotted against the logarithms of the products of time and concentration. Then an attempt was made to draw a set of the best fitting parallel straight lines through all the points representing the observations on one concentration at different times (lines marked as –1, –2, –3, etc.). Thus the line marked –5 is visibly the best straight line through points 5–5, 6–5, 8–5 representing observations made on concentration No. 5 (0·01 per cent.) at different times. These lines represent the probit line connecting log. time and probit mortality at different concentrations. Similarly another set of parallel straight lines 2–, 3–, 4– have been drawn as visibly the best fitting parallel straight lines through points representing observations made on different concentrations at the same time. Thus the line marked as 3– represents the best free-hand line through points 3–1, 3–2, 3–3, etc., *i.e.*, the data collected at different concentrations at the time of the third observation. These lines represent the relation between log. concentration and probit mortality at the time of different observations. The plane represented by these two sets of parallel lines is called the probit plane, showing the combination of time- and concentration-effects on mortality.

It will be clear from fig. 3 that it has been possible to draw with some justification 6 out of 8 possible parallel lines (–1 to –6) representing the log. time-probit mortality

relation but it has *not* been possible to draw with any justification more than 3 out of 17 theoretically possible lines representing log. concentration-probit mortality relations. Much more work may be needed to understand fully the actual meaning of these differences. With our present knowledge it can only be concluded that in this experiment time-spacing has been better than concentration-spacing.
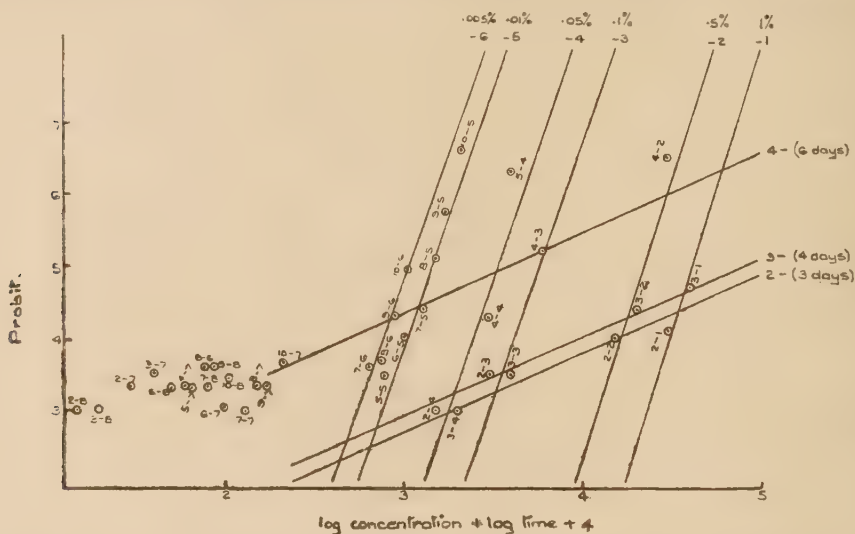
Fig. 3.—*Probit-plane.* Probit against log. time+log. concentration in marking points like 3–4, 4–3, etc., the first figure represents the serial number of observation, and the next, the serial number of concentration.

Also it is clear from fig. 3 that the points are much nearer to the set of lines –1 to –6 (log. time-probit mortality) than to the other set 2– to 4– (log. concentration-probit mortality). This difference is obviously due to the fact that points on the lines –1 to –6 are not independent as they represent observations on the same batch of insects at different times, whereas the points on the lines 2– to 4– *are* independent representing observations on parallel batches of insects. Thus, while the latter set (2– to 4–) includes the inherent variation from batch to batch, the former set is free from this variation. From the statistical point of view the former set is certainly inferior, as in it certain information regarding variation from batch to batch is lacking, but for observing the delicate trends of relations between any two factors, the lower the variations the better. The data, however, do not warrant any attempt to fit a formula to the probit plane relationship.

Average survival period on films of different strength. In the curve A of fig. 4, has been plotted the relation between log. concentration and the average survival period. The reciprocal of the survival period multiplied by 100 was taken to be the speed-index of toxic effect—$\left(\dfrac{1 \times 100}{\text{Average survival period}}\right)$. The average survival period has been calculated by finding a weighted mean of the survival period of all the insects on the films of one concentration (Table II). Thus on the films of 1 per cent. concentration 19 per cent. died between observations I and II (columns I and II), *i.e.*, after 1 day and before 3 days. Hence the survival period of these 19 per cent. was taken to be $\dfrac{1+3}{2} = 2$ days. On the same concentration another 19 per cent. died between observation II and III (columns II and III), *i.e.*, after 3 days and

TABLE II.

Survival of T. castaneum adults on DDT films of different strengths.

Column	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	Average survival period in days	100 ÷ Survival period
Survival period in days	·5	2	3·5	5	7	9	11·5	14	16	19	22	24	26	28	30	32·5	35		
Conc. gm./100 cc. sprayed									Per cent. Survival										
1·0	0	19	19	62	0	0	0	0	0	0	0	0	0	0	0	0	0	4·15	24·2
0·5	0	16	11	66	7	0	0	0	0	0	0	0	0	0	0	0	0	4·5	22·2
0·1	0	7	0	53	40	0	0	0	0	0	0	0	0	0	0	0	0	5·6	17·9
0·05	0	2	0	24	65	9	0	0	0	0	0	0	0	0	0	0	0	6·6	15·6
0·01	0	0	0	0	9	13	12	21	24	16	5	0	0	0	0	0	0	14·1	7·1
0·005	0	0	0	0	0	0	12	2	13	27	9	4	9	7	14	3	0	21·3	4·7
0·001	0	5	2	0	0	0	0	2	0	11	2	9	18	23	15	10	3	25·1	4·0
0·0005	0	2	0	0	0	7	0	3	0	5	12	4	20	15	12	13	2*	25·3	3·9
Sc	0	0	0	2	0	3	0	0	0	6	5	18	18	16	21	4	7	26·14	3·8
C	0	0	0	0	0	0	5	2	5	19	7	5	19	12	12	12	2	24·5	4·1

*5 survived at this stage but they have been taken to have survived only 35 days.

before 4 days. Hence the survival period of these other 19 per cent. was taken to be $\frac{3+4}{2}=3\cdot5$ days. Similarly the survival period of the rest (62 per cent.) was 5 days. Hence the weighted average of the survival period on the film of 1 per cent. concentration was $\frac{(19\times2)+(19\times3\cdot5)+(62\times5)}{100}=4\cdot15$ days. Similarly the survival period on other concentrations was calculated (Table II).

The curve B of fig. 4 represents the relation between log. concentration and the reciprocal of survival period. This has been drawn in the hope that in future it may be found practicable to regard the reciprocal of survival period as an index of the speed of toxic action. These curves are similar to the well-known curves representing the relation between temperature and longevity or developmental index of insects.
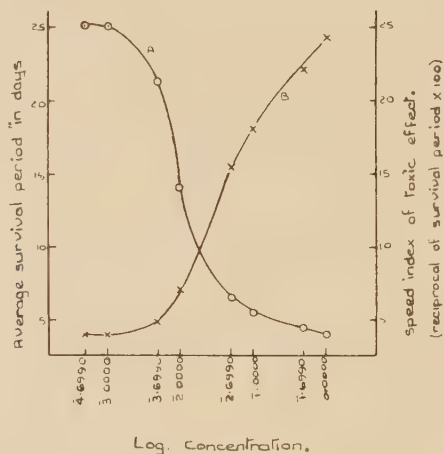
Fig. 4.—Average survival period plotted against log. concentration when *T. castaneum* adults are kept confined without food on DDT films.

Concentration-Time-Mortality Relation between γ–BHC and *T. castaneum*.

This study was on the same general lines as that on DDT and *T. castaneum* except that the insects were confined on the films within glass cones and not within glass funnels as such.

20/v/46. Twenty-seven filter papers were sprayed with 0·05 per cent. C.H.D.S. containing varying concentrations of the γ isomer of B.H.C. (3 at each concentration) in a Potter spray tower; 5 cc. of the emulsion was sprayed on each filter paper at 18 cm. pressure. The sprayed filter papers were left in their respective dishes and kept in the C.T. room at 80°F. to dry overnight.
21/v/46. Fifteen insects were enclosed on each filter paper and three replications were made at each concentration.

As a toxic effect was noticeable after 2 hours' contact with the film, 4 observations were made within less than 24 hours, and 2 further observations were taken at intervals of less than a day. These 6 inspections were made in the C.T. room without transferring the insects to the warm C.T. plate. The rest of the 25 observations were made on the C.T. plate as usual. After each examination, the dead insects were thrown away and the living were enclosed on their respective films for the next inspection.

The relation of percentage mortality on films of different concentrations during different inspections are given in Table III. This table has been prepared in the same way as Table I.

TABLE III.

Percentage mortality of *T. castaneum* adults after contact with γ-BHC films of different strengths.

Column	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI
Contact period in days	0·1	0·2	0·3	0·9	1·1	1·9	3	4	6	7	8	9	10	12	14	15	16	20	22	27	30
Conc. Control (sprayed)	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	8	12	27	41	65	82
0·0019	0	0	0	0	0	0	2·2	2	2	2	2	2	2	9	14	16	23	47	56	74	84
0·0078	0	0	0	0	0	2·2	7	9	11	11	11	11	16	21	25	25	27	52	59	73	80
0·031	0	0	0	0	0	29	42	73	8	87	87	87	87	91	96	96	96	100	100	100	100
0·125	0	0	0	29	64	76	89	98	100	100	100	100	100	100	100	100	100	100	100	100	100
0·25	0	11	27	67	84	93	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
0·5	0	49	71	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1·0	0	76	87	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2·0	0	53	76	91	93	96	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Concentration-mortality relation. The relation between percentage mortality and log. concentration has been plotted in fig. 5. A comparison of this figure with fig. 1 does not reveal any essential difference between the actions of DDT and γ-BHC. In view of the difficulties met with in the case of DDT, no adjustment was made in this case for deaths in the control. As in fig. 1, the shape of the curves in fig. 5 varies from a horizontal straight line showing no death at any concentration to a horizontal straight line showing 100 per cent. death at all concentrations. The experiment was not continued till all died, but the 21 curves are enough to show that the trend is the same as in fig. 1.
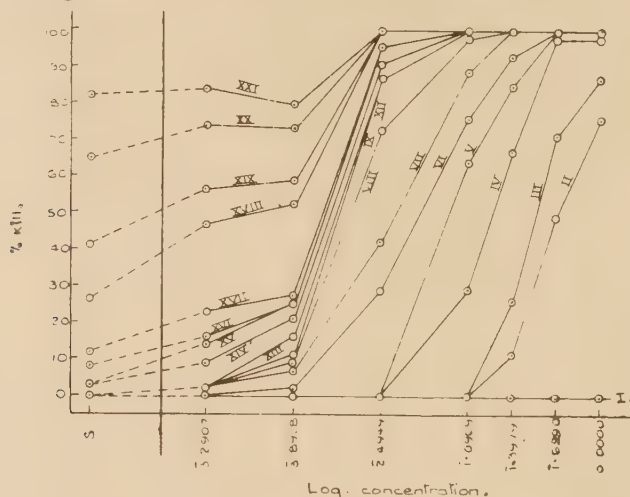
Fig. 5.—Per cent. mortality-log. concentration relation after various periods of contact between *T. castaneum* (adults) and films of γ-BHC.

Time-mortality relation. The time-mortality relation is plotted in fig. 6 and is essentially similar to fig. 2.
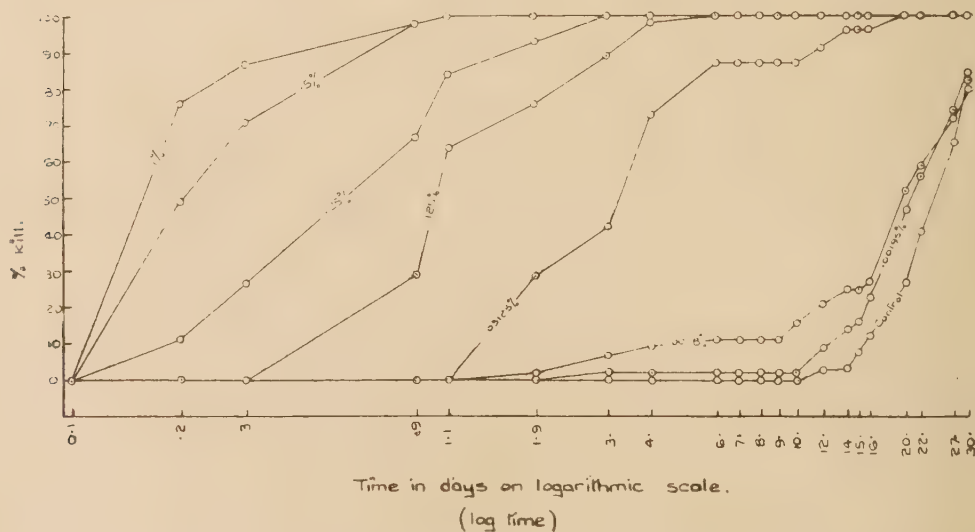
Fig. 6.—Per cent. mortality-log. time relation when *T. castaneum* adults remain in contact with γ-BHC films of various strengths.

Concentration-time-mortality relation. In view of the essential similarity between DDT and γ-BHC both in concentration-mortality and in time-mortality relations, probit planes were not plotted since it is certain they would be essentially similar to fig. 3.

Average survival period on films of different concentrations. In view of the promising nature of the graphs shown in fig. 4, the data of Table III were converted so as to give the average survival period and speed index of toxicity at different concentrations. These values are plotted in fig. 7, which should be compared with fig. 4. As the average survival period in this case varied from 0·22 days to 23·17 days it was difficult to plot this range on the same graph. Hence, only the higher 6 concentrations are plotted in fig. 7. At first sight this figure appears to be totally different from fig. 4 but the difference is probably due to the enlarged scale of the ordinate necessary for showing the quick change taking place at the higher concentrations. In other words, the difference is due to the much quicker action of γ-BHC as compared with DDT.
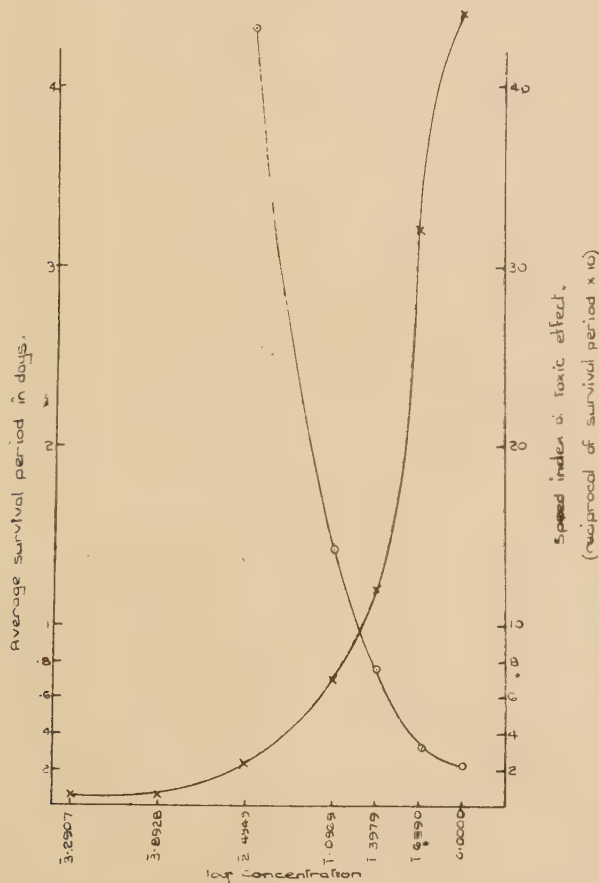
Fig. 7.—Average survival period and speed index of toxic effect plotted against log. concentration when *T. castaneum* adults were kept confined without food on γ-BHC films.

Concentration-Time-Mortality Relation between DDT and Larvae of *Plutella maculipennis*.

There is an important difference between the behaviour of *T. castaneum* adults and *Plutella maculipennis* larvae. Unlike the former, the latter crawl up the side of the glass cones ; hence they had to be confined over the film under a filter paper cone the inner surface of which carried a similar insecticide film.

22/vii/46. Six filter papers were sprayed with each concentration (see p. 12) and kept in the C.T. room to dry overnight.

23/vii/46. Three filter papers sprayed with each concentration were converted into equal cones with film surface inside and 10 *Plutella* larvae were enclosed on each film under the respective cones. They were kept in the C.T. room.

The first set of observations made after 24 hours' contact, on 24/vii/46, are given in Table IV.

TABLE IV.

Toxic effect on larvae of *P. maculipennis* of 24 hours contact with DDT films of various strengths.

Concentration gm./100 cc. sprayed	Percentage		
	Dead (D)	D+M	D+M+B
Control	0	0	0
0·0078	0	0	0
0·0156	0	0	0
0·0312	30	37	37
0·0625	43	43	77
0·125	40	50	80
0·25	87	93	97
0·5	57	70	93
1·0	80	93	100

TABLE V.

Deaths due to toxic effect of DDT film, and cannibalism caused by starvation, among larvae of *P. maculipennis* after 48 hours.

Replication	I				II				III			
Concentration gm./100 cc. sprayed ...	D	M	N	C	D	B	N	C	D	B	N	C
Control	—	—	8	2	—	—	9	1	—	—	9	1
Sprayed control ...	—	—	9	1	—	—	10	—	—	—	10	—
0·0019	—	—	9	1	—	—	6	4	—	—	10	—
0·0039	—	—	5	5	—	—	4	6	—	—	10	—
0·0078	—	—	6	4	—	—	5	5	—	—	9	1
0·0156	—	—	3	7	—	—	5	1	1	1	5	3
0·0312	9	1	—	—	8	1	1	—	10	—	—	—
0·0625	10	—	—	—	9	1	—	—	8	2	—	—
0·125	10	—	—	—	9	1	—	—	10	—	—	—
0·25	10	—	—	—	10	—	—	—	10	—	—	—
0·5	8	2	—	—	10	—	—	—	10	—	—	—
1·0	10	—	—	—	10	—	—	—	10	—	—	—

NOTE.—Food was given in replication III after first 24 hours and note less cannibalism in that replication (C.).

D=dead, M=moribund, B=badly affected, S=slightly affected, N=normal, C=killed or eaten (cannibalism).

After the first observation, the effect of starvation was significantly noticeable from the general conditions of the surviving larvae. Hence food was given to replicate III of each concentration. The next day's observation revealed (Table V) the violent reaction from starvation. Cannibalism was apparent beyond doubt since in replication III where food was provided this was much less noticed. It is difficult, therefore, to analyse the purely toxic effect of the insecticide. Observations, however, were continued.   All, except one in concentration 0·0312 per cent., were dead or badly affected in concentrations above 0·03 per cent. but below this the toxic effect could not be separated from that of cannibalism. The effect of concentration, however, became obvious in the numbers that pupated ultimately (fig. 8).
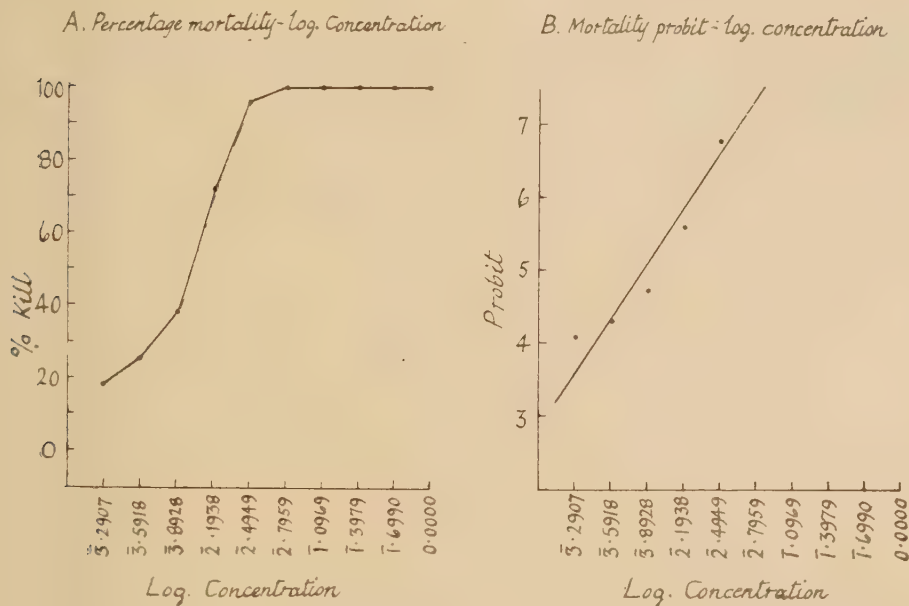
Fig. 8.—Toxicity of DDT films to larvae of *Plutella maculipennis* (inspection continued till pupation of surviving larvae).

Concentration-Time-Mortality Relation between γ-BHC and Larvae of *Plutella maculipennis*.

For this study the γ-BHC films were made on circles of bolting silk instead of filter paper and two circles were sprayed for each replication. One of the circles was converted into a cone with film surface inside and insects were enclosed on the other circle under the corresponding cone. The bolting silk was substituted for filter paper in order that its mesh structure should reduce the fumigation effect of γ-BHC. Cannibalism was, however, too marked with these insects to permit of any gradation of toxic effect with concentration.

Concentration-Time-Mortality Relation between DDT Film and *Macrosiphoniella sanborni*.

Macrosiphoniella sanborni proved to be rather highly susceptible both to environmental conditions and to DDT. The first few trials carried out in May 1946 ended in complete failure because none died within the first 24 hours and all including the control died during the second 24 hours. As they were enclosed within filter

paper cones, their course of reaction could not be traced and, being kept in a constant temperature room at 80°F., it was suspected that such a temperature did not provide a suitable environment. Hence, separate lots of 15 Aphids each were kept without food (*a*) in the glass house insectary, (*b*) in the C.T. room and (*c*) in a basement room at approximately 58°F. It was found that in the C.T. room all died on the third day, in the insectary they survived for 4 days and some survived up to 6 days in the basement room.

The second experiment with DDT on *M. sanborni* was carried out in August 1946 at room temperature. Five concentrations from 1 per cent. to 0·062 per cent. were sprayed and films were made on bolting silk instead of filter paper so that the course of reaction could be partially observed without disturbing the insects.

The toxic effect became visible within 15 minutes and within half an hour all appeared to be affected ; within 3½ hours all were badly affected at all concentrations. Any graduation of effect due to concentration could, however, not be observed ; probably all the concentrations were too high. As the controls were normal the environment could not be regarded as a disturbing factor.

Examination without disturbing on the second day did not show any appreciable change. The third day's rigorous examination on the warm plate revealed that there was a gradation in the percentage values of dead Aphids but *all* were badly affected at all the concentrations.

The third experiment on DDT and *M. sanborni* was conducted in September 1946, and much lower concentrations of DDT were used.

9/ix/46. Spraying :—Between 3 and 4 p.m. ; temperature 60°F. ; relative humidity 75 per cent. ; pressure 19 cm. ; deposit in 9 cm. dish—0·6568 to 0·6884 gm. After spraying the dishes were kept in C.T. cabinet at 80°F. The spray medium was 10 per cent. benzene in water emulsion with C.H.D.S. (0·05 per cent.).

10/ix/46. In the morning cones were made from bolting silk sprayed the previous afternoon. Insects were enclosed on the films between 2.30 and 3.30 p.m. ; three replications with about 15 insects in each.

TABLE VI.

Observation on the toxic effects of DDT films on *M. sanborni*.

Concentration gm./100 cc.	2nd day obs. on 11/ix/46					3rd day obs. on 12/ix/46					
	D	M	B	S	N	D	M	B	S	N	Y
Control	0	0	0	0	48	0	0	0	0	48	200
Sprayed control	0	0	0	1	43	0	0	0	1	43	9
0·0005	0	1	1	4	37	2	2	2	9	28	21
0·001	0	0	1	5	39	0	0	2	10	31	21
0·002	0	0	0	9	36	3	0	4	13	25	0
0·004	0	0	0	21	8	4	0	6	11	8	2
0·008	0	1	4	21	19	7	4	16	15	3	0
0·016	0	0	26	22	0	3	8	31	5	1	0
0·031	0	0	42	8	0	*	—	—	—	—	—
0·062	0	0	47	0	0	*	—	—	—	—	—
0·125	0	0	44	0	0	*	—	—	—	—	—
0·25	0	2	42	0	0	*	—	—	—	—	.
	After inspection kept in food cages					After inspection kept in food cages					

D=dead, M=moribund, B=badly affected, S=slightly affected, N=normal, and Y= young nymphs reproduced during experiment.

Aphids confined on the films on 10/ix/46.

*Not examined, but there appeared to be no change in general condition and a fourth day's inspection showed no recovery.

Observations through the bolting silk without disturbing the insects confirmed those of the second experiment. After 2–3 hours all the insects in concentrations down to 0·062 per cent. were badly affected and a good many were badly affected even down to 0·016 per cent. concentration.

After the second day's examination the live Aphids were kept in specially prepared food cages which consisted of food leaves planted in moist sand and enclosed within glass cones. The sand was covered with filter paper. These food cages appeared to provide a fairly congenial environment as the Aphids began to reproduce. Counts on the third day's inspection (Table VI, col. Y) show that those on the unsprayed control (c) reproduced much more than others exposed to films of lowest concentrations of the insecticide.

The observation made on the second and third days are given in Table VI. Fig. 9A gives the percentage of insects dead, moribund or badly affected on the second and third days. The same data are also plotted in a probit log. concentration form (fig. 9B).
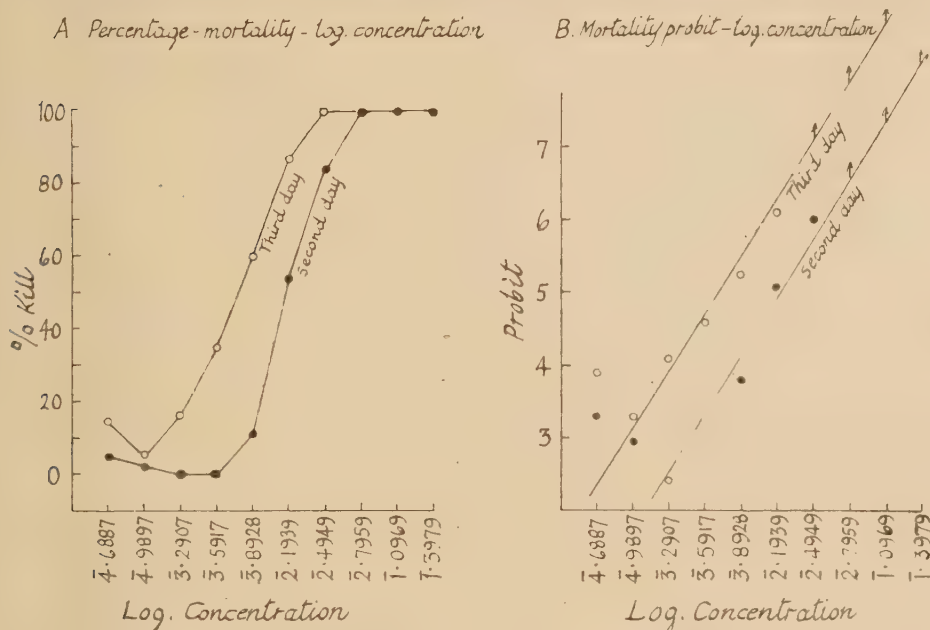
Fig. 9.—Toxicity of DDT films to *M. sanborni* (inspections after 24 hours' and 48 hours' contact).

Concentration-Time-Mortality Relation between γ-BHC and *M. sanborni*.

This experiment was carried out before the third experiment on *M. sanborni* and DDT. The idea of suitable food cages was not utilised therefore in this experiment.

28/viii/46. Spraying :—Between 3 and 5 p.m. ; pressure 18 cm. ; temperature 65–67°F. ; R.H. 70–67 per cent. After spraying the dishes were kept in C.T. cabinet at 80°F. to dry. Spray medium, 10 per cent. benzene in water with 0·05 per cent. C.H.D.S. emulsion.
29/viii/46. Insects enclosed, three replications with about 15 insects in each.

Observations through the bolting silk showed that within 4 hours all were badly affected at the high concentrations and various shades of effect were visible in lower concentrations but no quantitative record could be made. The observations based on a more careful inspection on the second and third days are given in Table VII ; those for the second day are graphed in fig. 10.

Side by side with this experiment the film of 2 concentrations (0·0625 per cent. and 1·0 per cent.) were tested only for fumigation effects. In these cases the Aphids were enclosed within clean bolting silk cones on perforated zinc plates which were kept separate from, but just above, the film. Above the films corresponding to 1·0 per cent. concentrations all were dead or moribund on the second day, and above the 0·0625 per cent. concentration the fumigation effect became quite clear on the third day.

TABLE VII.

Effect of γ-BHC films on *M. sanborni*.

Concentration gm/100 cc.	D+M+B after 24 hours' contact with film per cent.	D+M+B after subsequent 24 hours in separate tubes with food leaf per cent.
C	4	44
S	20	57
0·008	34	52
0·016	29	69
0·031	31	63
0·062	76	96
0·125	83	100
0·25	100	100
0·5	100	100
1·0	100	100

D=dead, M=moribund, B=badly affected.

A. Percentage mortality-log. concentration   B. Mortality probit - log. concentration
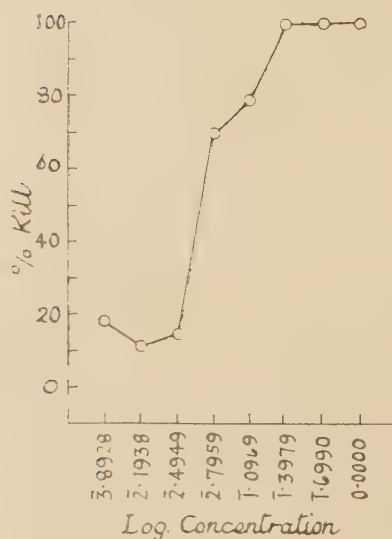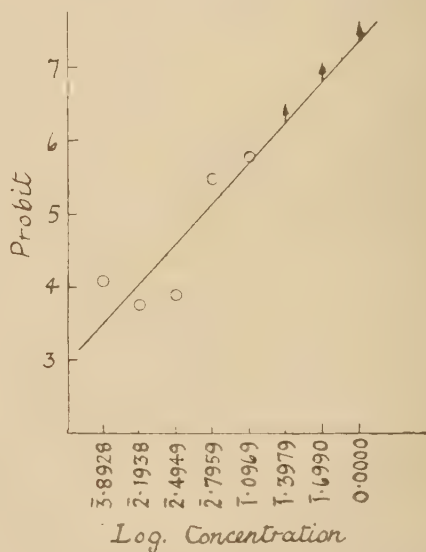
Fig. 10.—Toxicity of γ-BHC films to *M. sanborni* (inspection after 24 hours' contact).

Discussion.

The experiments described above showed that for fundamental work on the film technique *Tribolium castaneum* is the most suitable insect. The great advantage

of this species is that it can be kept in continuous contact with the film without difficulty. Hence, after these experiments it was decided that for any further exploratory work *T. castaneum* should be used, and other species were employed only to test whether conclusions arrived at with *T. castaneum* applied generally.

The significance of much of the work so far described can only be regarded as of a tentative nature, but it is of sufficient importance to warrant further and more detailed investigation. The log. concentration-percentage mortality curves changing shape, with time (figs. 1 and 5) can obviously be obtained with other species besides *T. castaneum*. Similarly with greater care curves connecting log. concentration and average survival periods can be obtained with other species (figs. 4 and 7).

Elimination of Fumigation Effect from Contact Effect of γ-Benzene Hexachloride.

γ-BHC is said to be essentially a contact insecticide but it is also known to have a fumigant effect. In order to gauge the significance of the latter in the film technique a simple experiment was carried out. To detect any feeble fumigant action a very strong film of γ-BHC was considered desirable in the first instance; 20 per cent. of γ-BHC in benzene was, therefore, poured into a petri dish containing a filter paper so that the solution flooded the whole filter paper, and the solvent then allowed to evaporate. The filter paper was removed and 15 insects were enclosed over the filter paper but not in contact with it. In less than 24 hours 7 of the 15 insects were dead and the rest were moribund. This experiment showed that the fumigation effect is pronounced. The question therefore arises whether it is practicable to eliminate this effect from that due to contact and if so to what extent, so as to be able to study practically the pure contact action. The following experiments were therefore undertaken.

1. Combination of contact and fumigation effect :—The combination of contact and fumigation action was studied by enclosing insects (*T. castaneum*) over the film by filter funnels, the top holes of which had been closed by plasticine.

2. The progressive elimination of the fumigation effect :—

(*a*) Confining insects over films with open truncated cones (Pl. 1b) ;

(*b*) Confining insects over films with truncated cones covered by wire gauze or perforated zinc covers (Pl. 1g) ;

(*c*) Confining insects over films with perforated filter paper cones. (The method is clear from Pl. I, fig. *e*) ;

(*d*) Confining insects over films within filter paper cones fitted with exhaust draughts (Pl. I, fig. *d*) ;

(*e*) Confining insects over films with perforated filter paper cones fitted with exhaust draughts (Pl. I, fig. *e*).

3. *Fumigation effect only.* Insects confined within an inverted crystallisation dish over muslin at a distance from the film (Pl. I, fig. *h*).

4. *No fumigation or contact effect.* Insects kept as in (3) but confined by open truncated cones (Pl. I, fig. *i*). This was arranged to ascertain whether the open cut cone eliminates all fumigation action. If so, this arrangement should give results of the same order as the controls.

All these arrangements were tried with films of 4 different strengths.

24/vi/46. Spraying. 5 cc. of each emulsion was sprayed on each filter paper ; 24 filter papers were sprayed with each concentration. With concentration No. 4 (2·5 per cent. solution) the film was made by flooding the filter paper in a dish of 9·7 cm. diameter using 10 cc. for each filter paper and not by spraying. The solvent was allowed to evaporate from the dish without removing the filter paper from it.

25/vi/46. Insects were enclosed under the various devices 26 hours later.

26/vi/46. Inspection was carried out 22–24 hours after enclosure. In devices (2c) to (2e) it was observed that the insects crawled up the cones. These cones would therefore need spraying in all future work with them.

S. PRADHAN.

The results are shown in fig. 11.

It was found that in this experiment the toxic effect was less in the case of 2·5 per cent. *solution* than a concentration of 0·5 per cent. used as an *emulsion*. This result needs confirmation if it is not to be ascribed to chance, but it may well be due to the form in which the toxic material separates out in the film.

The efficiency of the different arrangements for eliminating the fumigation effect is shown by fig. 11. As there is considerable toxic action even in arrangement No. 4 where there is no contact effect and the insects are confined by open cones the complete elimination of a fumigant effect in the case of γ-benzene hexachloride appears impossible and it was decided not to pursue the matter further at this stage.

XX = No experiments.
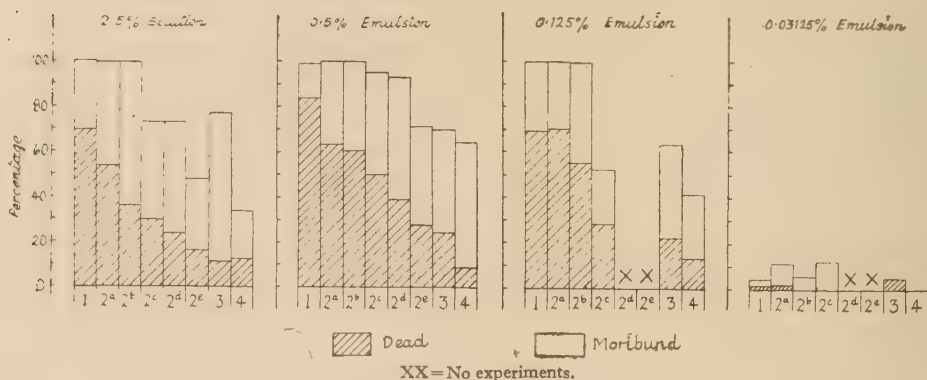
Fig. 11.—Elimination of fumigation effect from contact effect of γ-BHC.

EFFECT OF THE NATURE OF SURFACE ON THE TOXICITY OF INSECTICIDE FILMS.

This effect is obviously one of importance as it is known that the ease with which an insect can be killed depends on the surface upon which it is placed for spraying. The persistent residual effect of DDT sprays accentuates its significance. Eight kinds of surfaces including 3 kinds of leaf surfaces were tested both with DDT and γ-benzene hexachloride. *T. castaneum* was used as test insect. A crop pest, especially on leaf surfaces, would have been preferred but *T. castaneum* was adhered to because of the ease of keeping it in contact with the film. In view of the possibility of enclosing insects within cones of the same surface as that being tested any insect can be used with such surfaces as filter paper and bolting silk which can readily be converted into cones but the difficulty of making good cones from foliage finally led to the decision to retain *T. castaneum* as the test subject.

(a) *Effect of Surface on Toxicity of DDT Film.*

The following surfaces were tested :—

1. Glass surface—ordinary dish of 9 cm. diameter.

2. Wax surface—glass dish coated with wax.

3. Filter paper—Whatman No. 544 (9 cm. diameter).

4. Bolting silk.

5. Water lily leaf (*Nymphaea* sp.).

6. Marrow leaf (*Cucurbita pepo* var.).

7. Cabbage leaf (*Brassica oleracea* var.).

8. Geum leaf (*Geum* sp.).

Circles of 9 cm. diameter were cut from bolting silk and leaves of water lily, marrow and cabbage. The Geum leaf was used as such. All these surfaces were sprayed with an emulsion of the same concentration. After spraying, Nos. 1 to 4 were left simply to dry but the leaves were removed from the dish in which they were sprayed and, to prevent desiccation, were kept on moist filter paper which itself was kept on moist sand. After the medium had evaporated, insects were enclosed on the film for a definite period and examined.

4/x/46. Spraying. Between 3 and 5 p.m. ; pressure 19 cm. ; temperature 60–62°F. ; R.H. 80–84 per cent. ; deposit on dish of 9 cm. diameter—0·8030 to 0·7930 gm. After spraying the dishes were kept in the laboratory.

5/x/46. Insects enclosed between 12.30 to 1.30 p.m.
10/x/46. Inspection on warm plate.

The results are given graphically in fig. 12. Phytocidal effects were noticeable on leaves of water lily, marrow and cabbage.
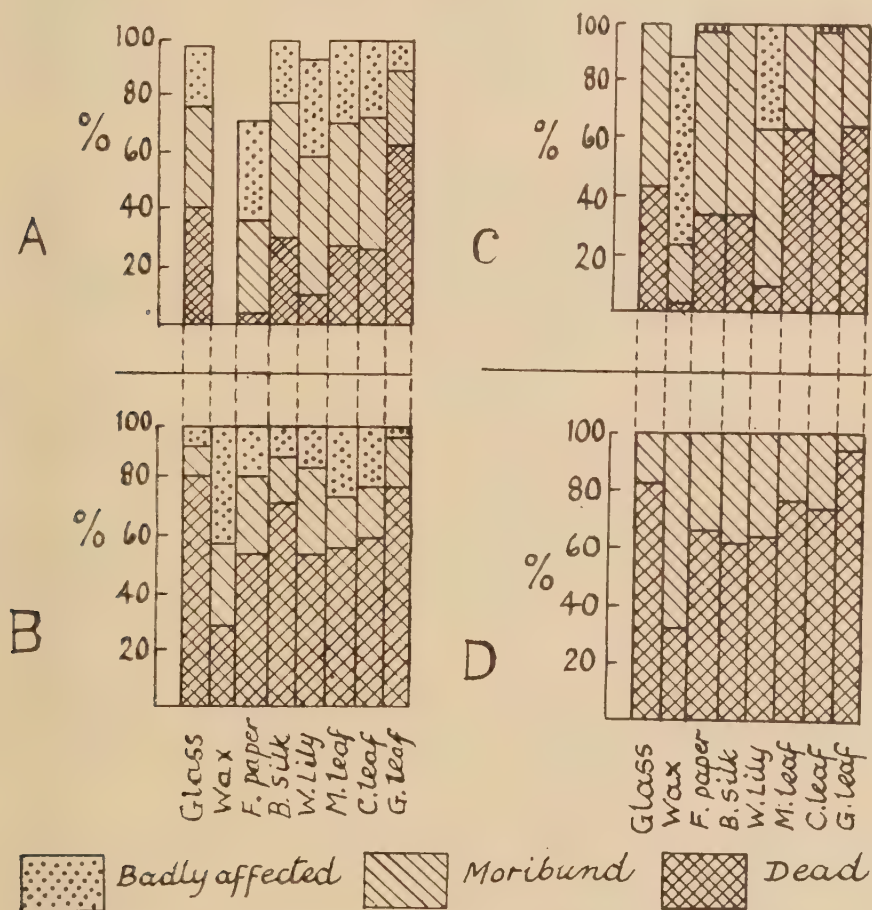
Fig. 12.—Effect of surface on toxicity of films of DDT and Y BHC.
A. 0·1 per cent. DDT. Exposure—5 days.
B. 1·0 per cent. DDT. Exposure—5 days.
C. 0·125 per cent. γ-BHC. Exposure—20 hours.
D. 0·5 per cent. γ-BHC. Exposure—24 hours.

(b) *Effect of Surface on Toxicity of γ-BHC Film.*

The same surfaces were tested as with DDT and the method of experimentation was also the same.

10/x/46. Spraying. Between 4.30 and 5.30 p.m. ; temperature 63–65 F. ; R.H. 60–65 per cent. ; pressure 19 cm. ; deposit in 9 cm. diameter dish—0·6040 gms. After spraying the surfaces were kept at lab. temperature as in the last experiment.

11/x/46. Insects enclosed between 12–1 p.m.

12/x/46. Inspection on warm plate between 11 a.m. and 1 p.m.

The results are given in fig. 12.

The following tentative conclusions can be drawn from the two foregoing experiments :—

1. Wax surfaces in all the four cases have shown least toxicity.

2. Geum has consistently given a high kill—highest in 3 out of 4 experiments— higher even than glass. It is possible that the hairy nature of the leaf helps retention of the spray fluid and in addition, effects contact with a larger area of the insect body.

3. Water lily leaf gives definitely less kill in lower critical concentrations than other surfaces (except wax) but at higher concentrations does not differ markedly from other leaves.

SUMMARY.

Experiments are described in which the concentration-time-mortality relation-ships with certain insects were investigated for the insecticides DDT and γ-BHC, *the insecticides being used in film form.* Adults of *Tribolium castaneum*, the larvae of *Plutella maculipennis* and adults of the Aphid, *Macrosiphoniella sanborni*, were used as test insects. *T. castaneum* proved the most suitable insect for experimental laboratory work on films, since it can be kept in continuous contact with them without difficulty.

A description is given of the techniques employed for *T. castaneum* and also for the other insects which are able to progress on perpendicular glass surfaces. Techniques are described for experiments in which attempts were made to eliminate as far as possible the fumigation effect of γ-BHC and to differentiate it from that of direct contact. The fumigation effect of this compound is considerable and it appears impossible to eliminate it entirely.

With *T. castaneum* the characteristic curves connecting log. concentration with percentage mortality varies with time of exposure, from horizontal lines showing on the one hand zero toxicity and, on the other, complete mortality at all con-centrations. Sigmoid curves are represented between these limits. The average survival period of *T. castaneum* adults gradually decreases with the strength of poison in the film.

The surface upon which toxic films are deposited was shown in preliminary experiments to have a definite bearing upon their effectiveness. Waxed surfaces showed least toxicity, and amongst a few leaves used Geum gave the highest and water lily, at lower critical concentrations, the least effect. High concentrations on water lily were little different from others in toxic action.

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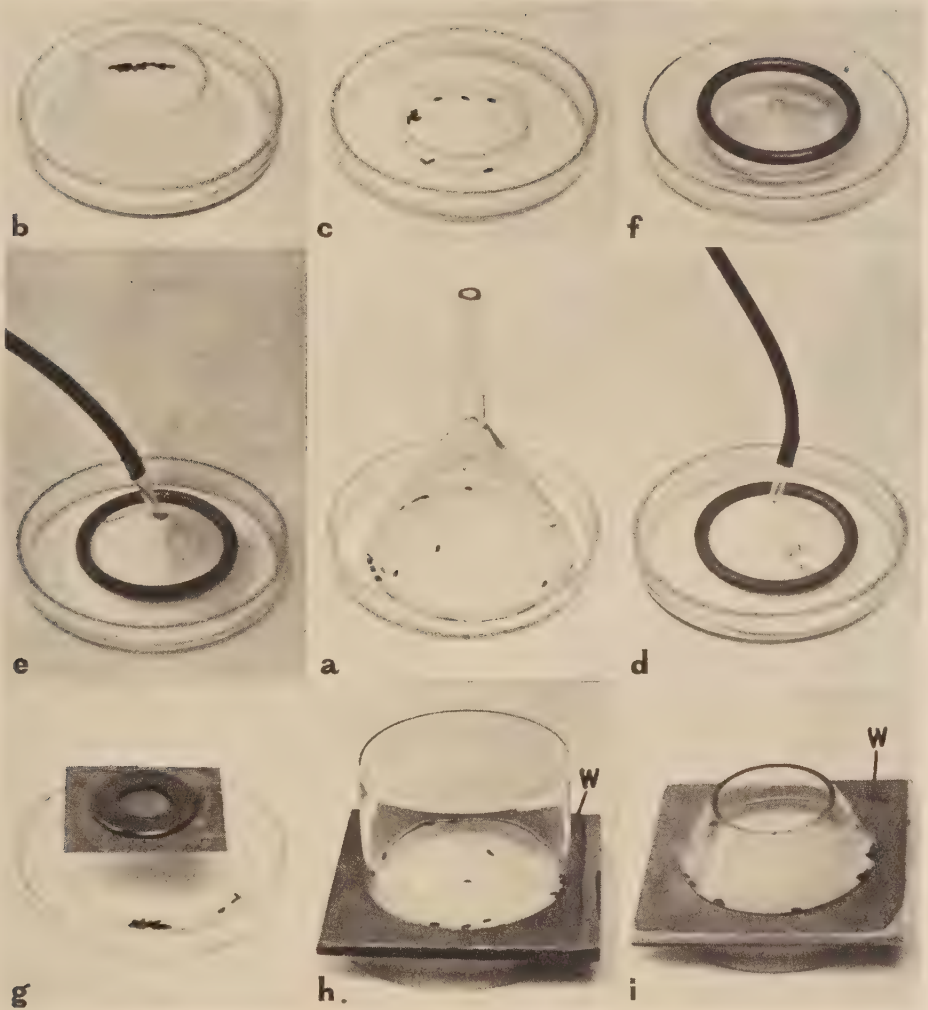
(a) *T. castaneum* confined over DDT film within filter funnel.

(b) *T. castaneum* confined over film within truncated glass cones.

(c) *T. castaneum* confined over film within glass ring.

(d) Filter paper cone fitted with a tube which can be connected with an exhaust device, pressed on a film with the help of an iron ring. This arrangement was tested for its efficiency in eliminating fumigant effect of y-BHC film from its contact effect.

(e) The same as (d) except that the filter paper was perforated with the help of a perforating die. Numerous pores can be noted in the photograph.

(f) Cone made of bolting silk pressed on the film by means of a glass ring which itself is weighted by an iron ring.

(g) Insects confined within truncated glass cone, covered by a perforated zinc plate pressed down by an iron weight.

(h) In this arrangement the insects are confined above but away from the film on filter paper placed on an inverted petri dish. Over the film is placed a wooden square (w) with a large circular hole in the centre, across which a piece of muslin is stretched and fixed by paste. The insects are confined over the muslin, within an inverted crystallisation dish. This arrangement was used for testing the fumigation effect of y-BHC films.

(i) The same as (h) except that the insects are confined within truncated glass cones. This arrangement was tried to ascertain if the fumigation effect of y-BHC film could be eliminated if insects were to be confined over the film as shown in (b).

LABORATORY BREEDING OF *ANOPHELES PUNCTULATUS PUNCTULATUS*, DÖNITZ.

By M. J. MACKERRAS and T. H. LEMERLE.
*Commonwealth Council for Scientific and Industrial Research.**

Anopheles punctulatus punctulatus, Dön., is an Australasian mosquito found in New Guinea, New Ireland, the Moluccas and the Solomon Islands, but not on the Australian mainland. This species and *A. punctulatus farauti*, Lav., are the principal vectors of malaria in the Australasian region.

During the war, an extensive study of the chemotherapy of experimentally transmitted malaria was undertaken at Cairns, North Queensland, by a special Army unit (the L.H.Q. Medical Research Unit), which was organised by Brigadier N. Hamilton Fairley. Large numbers of infected Anophelines were required for this work. At first, local species were used, but *A. punctulatus punctulatus* proved superior (Mackerras & Roberts, 1948), and arrangements were made to bring in regular consignments of larvae by air from New Guinea. When supplies from this source proved inadequate towards the end of 1944, it became necessary to establish a laboratory colony of sufficient size to provide the hundreds of young female mosquitos that were required daily.

There is a considerable literature on laboratory colonies of Anophelines, some of which have now been maintained for many years. Success has been obtained with " wild " as well as " domesticated " species, for example : Boyd, Cain and Mulrennan (1935) with *A. quadrimaculatus*, Say, in the United States : Hackett and Bates (1938) with *A. elutus*, Edw. (=*sacharovi*, Favr), other members of the *maculipennis*, Mg., complex, and *A. superpictus*, Grassi, in the Albania laboratory, and with *A. pharoensis*, Theo., and *A. multicolor*, Camb., in Egypt ; Mohan (1945) with *A. fluviatilis*, James, in India ; Russell and Mohan (1939) with *A. stephensi*, List., in India ; Rozeboom (1936) with *A. albimanus*, Wied., in Panama ; and Shute (1936) with *A. maculipennis atroparvus*, van Thiel, in England. Little of this information was available, however, when the work at Cairns was started, so that the methods had to be developed *de novo*.

The present paper describes the methods of breeding and handling that were evolved, and also records observations on the life cycle and behaviour of *A. punctulatus punctulatus†* under laboratory conditions.

Accommodation of the Culture.

(1) *The breeding room* (fig. 1).

The colony was housed in a rectangular room forming one corner of the entomological laboratory. The room was 22 feet long by 8 feet wide, with walls 8 feet high. The floor was of concrete built at ground level.

The room had a northern and western aspect, and was designed to give the best possible conditions of natural lighting within the limits of the available space. There were six sash windows each measuring 3 feet by 2 feet opening on the outside of the building, four of them in the wall facing west, and two in the wall facing north. In addition, there were two similar sash windows opening into the adjoining room.

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*The work here reported was carried out while the authors were officers of the Australian Army Medical Corps.

†Hereinafter, for brevity, simply called *punctulatus*.

The walls and ceiling of the room were lined with '' Caneite '', which insulated it to some extent against extremes of temperature. In fig. 2, daily readings of the maximum, minimum and mean temperatures are plotted for the months of June–July,
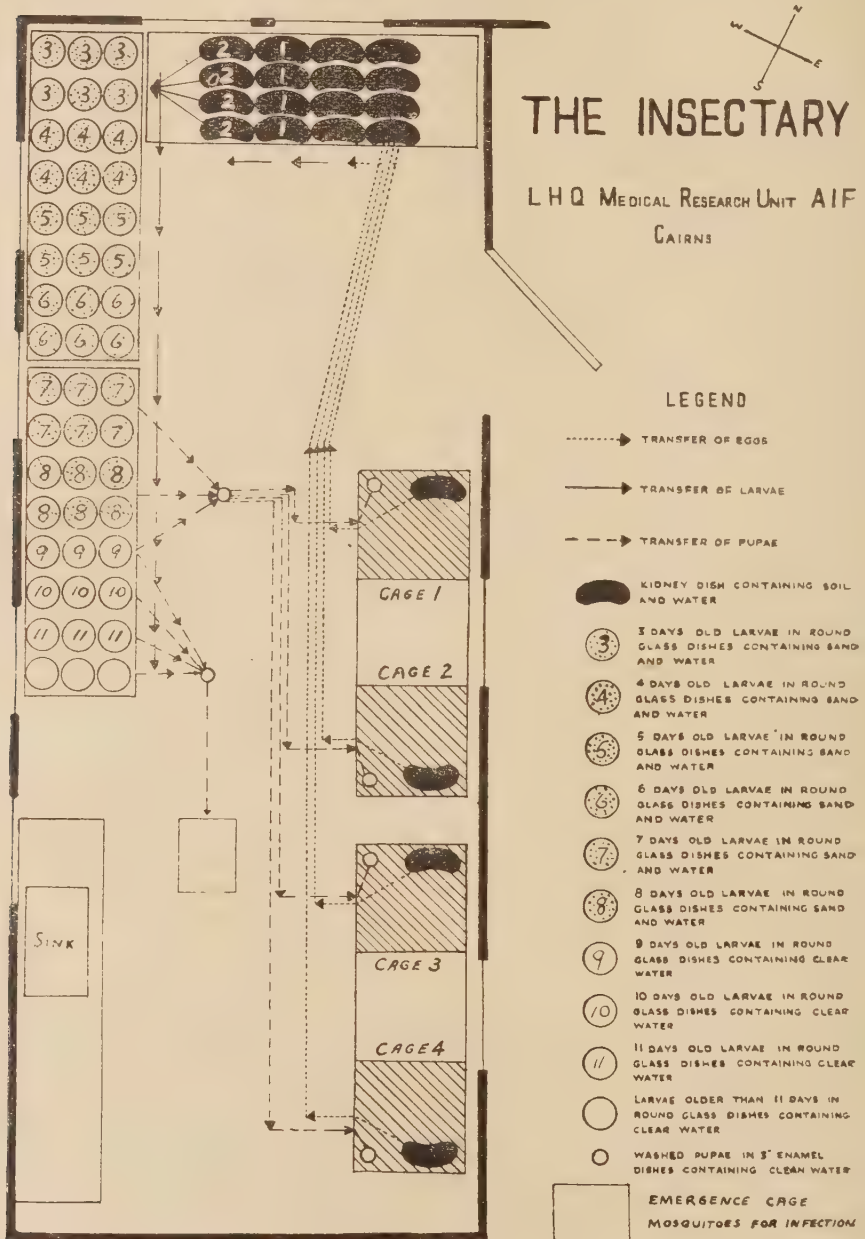
Fig. 1.—Plan of breeding room.

and November–December.   Readings for the two latter months represent summer temperatures in the breeding room, when the windows were left open throughout the day and the radiator was never used.   June and July are winter months, during which the windows were always closed, and one or two radiators switched on when necessary to maintain the temperature at approximately 27°C.
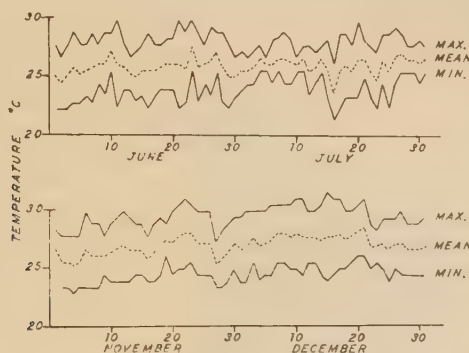
Fig. 2.—Maximum, minimum and mean temperature readings for June and July (winter), and for November and December (summer).

During the summer, direct sunlight penetrated into the room for a few hours in the afternoon through the windows in the western wall.   In winter it penetrated through the windows in the northern wall during most of the day.   It never reached the breeding cages, but fell on the dishes of larvae for varying periods during the day, depending on the time of year.

In the breeding room, the adults of the colony were kept separate from the aquatic stages.   The adults were accommodated in four large breeding cages, which were placed on two ant-proofed tables close to the eastern wall of the room. Each table was 6 feet by 2 feet by 3 feet high, and so placed that it was near one of the windows opening into the adjoining room (fig. 1).

The eggs, larvae and pupae of the colony were accommodated on three similar high tables, also ant-proofed, and placed close to the northern and western walls.

In addition, the room contained a sink, with water taps and a 7-foot draining board, for cleansing breeding dishes and apparatus.   Surplus dishes were stored under the tables carrying the larvae, and cages of emerging adults were accommodated on ant-proofed racks under the tables on which the breeding cages were kept.

(2) *The breeding cages* (fig. 3).

Adults of *punctulatus* require space, particularly during mating and oviposition, both of which processes are performed while the insects are in flight.   In addition they require a high humidity, and a rhythm of light conditions corresponding to night and day.   These needs were met satisfactorily by the breeding cages to be described.

These cages were 3 feet high and 2 feet square at the base.   Cages 4 feet high were tried, but proved less satisfactory ; they gave no better results, were unwieldy to clean, and it was difficult to observe the whole of the interior.   The cages had a wooden frame, with two glass sides, and the two other sides made from sheets of " Masonite " (compressed wood) : the roof and floor were half-inch timber.   During cleaning, the roof, which was made in one piece, could be removed, and the two glass sides could be slid out of grooves in the framework.

When a cage was in use, the only access to the interior was through three circular holes cut in the " Masonite " sides and covered with mosquito-net sleeves. In one side, 12 inches below the roof, there was a hole 3 inches in diameter and another 6 inches in diameter placed level with the floor. The top one was generally used for inserting an arm when the insects were given a blood meal, but also provided ventilation. The larger hole at the bottom was used for replacing the apple and oviposition dish, returning pupae to the cage, and removing dead adults. A third hole, for ventilation purposes only, was cut 6 inches from the roof of the cage in the side facing the wall of the room.
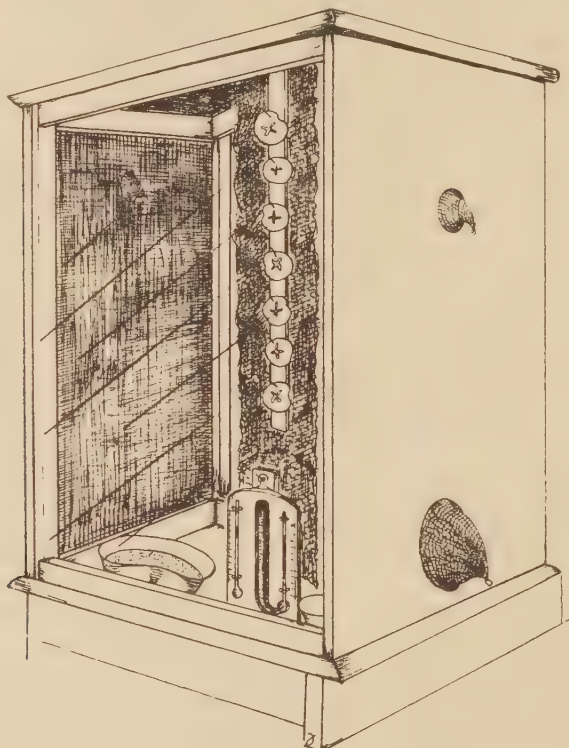
Fig. 3.—A breeding cage.

A strip of grey blanket 8 inches wide was hung from the centre of the roof, parallel to the glass sides and reaching to within a few inches of the floor. The top of this blanket was divided into three strips, each of which passed through a small hole in the roof of the cage and dipped into a 12-inch kidney-dish of water on top of the cage. The blanket was thus kept continuously moist by capillarity, the water dripping from its bottom being caught in a gauze-covered kidney-dish, and drained out every day with a siphon tube. The flow of water could be regulated to some extent by removing one or more of the strips of blanket from the dish on top of the cage. Usually one filling of the kidney-dish was sufficient to keep the blanket moist all day.

The strip of moist blanket provided damp daytime resting places for the adults. In addition, it assisted in maintaining a high humidity in the breeding cage. In fig. 4, daily 9 a.m. readings of relative humidity are plotted for the room and one breeding cage. Relative humidity in the breeding cage was maintained constantly at a much higher level than in the breeding room.

Also suspended from the roof of the cage was a strip of wood about 18 inches long, provided with twelve, evenly distributed spikes, on which slices of fresh apple were impaled. This stick could be detached from the roof, and removed from the cage through the large hole in one side, to enable fresh slices of apple to be affixed.

On the floor of the cage was nailed a small stand for a wet- and dry-bulb thermometer. It is appreciated that this was an unsatisfactory type of instrument for this kind of work, but small hair or paper hygrometers were not available at the time.
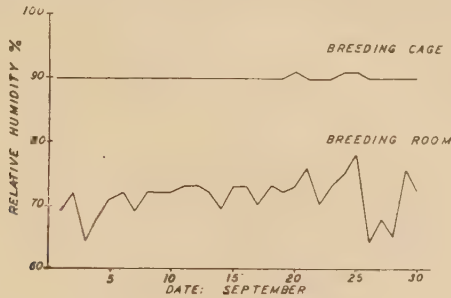
Fig. 4.—Relative humidity in breeding cage and in room, taken at 9 a.m. daily.

There were four breeding cages, each containing 12 cubic feet of space, and each capable of accommodating 2,000 adults without overcrowding (approximately 10 cubic inches per mosquito). At least one cage was in routine use all the time, the others being used for experimental work and as substitutes during cleaning operations. The limiting factor in production capabilities of the colony was space for rearing larvae, not accommodation for breeding adults.

Daily Routine of Management.

Care of adults.

Dead adults were removed from the floor of the breeding cages every morning, and, when the cage was being run as a longevity trial, the number and sex of the dead were recorded. The kidney-dish underneath the blanket was emptied, and fresh water added to the dish on top of the cage. The oviposition dish was replaced by a fresh dish.

Fresh slices of apple were introduced in the evening, so that the surface would be fresh and moist when the mosquitos began to feed during the night. When the apple was changed in the morning, the cut surface was found to become too dry by nightfall.

The blood meal was also given at night, when the females were normally seeking it. Although tedious and somewhat painful, it was more satisfactory to allow the females to take their blood meal from the arm of a human volunteer than from an animal. The 3-inch diameter hole fitted neatly round a human arm, and there was little danger of damage to the insects during feeding. The method was cleaner and less troublesome than if an animal had been placed in the cage each day.

Care of larvae.

The essential feature of larval management was that the age groups of eggs and larvae were kept separate until pupation was almost complete. The eggs laid in the breeding cages on one night, and the larvae which hatched from those eggs, represented a batch, which was kept distinct from batches laid on other nights. Every batch occupied its appropriate position on the tables, according to its age, and

every day the various batches were moved one position along the tables. In this way the exact age of a dish of larvae could be determined by its position on the tables, and any variations from the normal rate of development could be readily observed.

Twelve-inch, white enamel kidney-dishes containing soil and water were used as oviposition dishes. They exposed a large surface area of water to the insects, and allowed space for manoeuvre during ovipositing flights. Every day the oviposition dishes containing eggs laid the previous night were removed from the breeding cages in use, and placed in their appropriate positions on the table (fig. 1). The dead adults were removed from the surface of the water, in an attempt to minimise the chances of larvae becoming infected with any parasitic or pathogenic organisms harboured by the adults.

Until they were three days old, the larvae were kept in the dishes in which the eggs were laid. This obviated the need to handle eggs and first instar larvae, and enabled a check to be kept on the larvae obtained from a particular breeding cage. When three days old, all the larvae were bulked together, and redistributed into six circular dishes containing sand and water. These dishes were glass soup plates, $7\frac{1}{2}$ inches in diameter and $1\frac{1}{2}$ inches deep, and, when almost full, they exposed 35 square inches surface area of water. They were particularly convenient, for they were easy to clean and easy to move from place to place.

The larvae were transferred by means of a fine gauze scoop, through which the water containing them was poured. They were trapped as the water passed through, and the approximate number required floated off into each of the glass dishes.

At intervals throughout the day, the larvae were fed with " Farex ",* a proprietary brand of infant food, supplied in tins as a fine powder. A small quantity was rubbed between finger and thumb, and allowed to drop on the water, where it spread evenly over the surface, and, being light, would float for a considerable time. The quantity applied and frequency of application depended on the number of larvae in the dish. The aim was to supply as much food as possible, without over-feeding to the extent that the larvae became bloated and died, or the water became fouled by unconsumed food. When the larvae were overcrowded, they were best fed frequently with small quantities, rather than with large amounts at one time. About 300 larvae per dish was the optimum concentration from the point of view of ease in keeping a balanced food supply, but in case of need up to 1,000 per dish could be reared.

Pupation usually began when the larvae were six or seven days old, the pupae being removed every day with a wide-mouthed medicine dropper. When eight days old, and pupation was at a maximum, each batch was transferred to three dishes of clean water to facilitate removal of pupae. The water from the original dish was decanted off the sand through a 7-inch glass funnel on to a mosquito net sieve, the larvae and pupae were caught on the netting, and floated off into clean water.

Care of pupae.

The pupae recovered each day were washed thoroughly by catching them on a mosquito net sieve and passing a gentle stream of clean water over them. They were then floated off the sieve into 3-inch diameter enamel bowls containing clean water, usually 1,000 pupae to a bowl of 7 square inches surface area. The pupae almost covered the surface of the water, and no floatage was supplied to assist the adults during emergence. The emergence rate of adults from these dishes varied from 95 per cent. to 100 per cent.

*Farex—Carbohydrate 75·0 per cent., Protein 14·0 per cent., Fat 3·0 per cent., Minerals 4·0 per cent., Moisture 4·0 per cent.

The number of pupae returned to the breeding cages each day varied with production requirements. These were selected from batches of larvae which were just starting to pupate (Table I), while pupae from batches of older larvae were placed in smaller mosquito net cages, in which the adults were kept.

Observations on Life Cycle and Behaviour.

The egg.

The eggs of *punctulatus* are creamy white when first laid, but become uniformly black after about two hours in contact with water. Normally they hatched during the afternoon, about 42 hours after being laid. Apart from its value as a preferred oviposition site (see later), muddy water was advantageous to the eggs, in that the debris on the surface prevented them from being drawn to the sides of the dish and stranded as the water evaporated.

When laid on dry paper and kept dry for a period of 12 hours, the eggs remained creamy white in colour and did not hatch when placed in water. Some batches laid on water and allowed to change colour were dried out in the air, and stored for $5\frac{1}{2}$ days without affecting hatching. These eggs hatched within 2 or 3 hours of being placed in water. Other batches, however, treated similarly failed to hatch, so it would appear that some special conditions may be necessary, which were not appreciated at the time. Nevertheless, the fact that hatching may occur after a period of desiccation explains the extremely rapid appearance of larvae after rain in freshly filled ruts and depressions, a phenomenon which was observed quite frequently in New Guinea.

The larva.

Several conditions were found to influence larval development, of which the most important were depth, substrate, food, and temperature.

It had already been found that the larvae thrived best in shallow water, and the glass soup plates had been adopted as standard equipment before the present work began.

As regards substrate, it was at first considered essential to rear the larvae in water containing soil. Subsequently, sand was substituted for soil during the latter part of larval development, from 3 to 8 days, and the routine was standardised as already described. This was cleaner than soil, and appeared to absorb waste products and unconsumed Farex quite satisfactorily. Soil, on the other hand, was probably advantageous in the early stages, for it assured the young larvae of their mineral requirements and a bacterial scum on which to feed, as well as possibly acting as an absorbent for waste products.

In the field, *punctulatus* larvae are frequently found in water free from detectable algal growth, and no plant association, such as Shute used for *A. maculipennis atroparvus*, was found necessary in the laboratory. On the other hand, the natural food (probably bacterial) in the muddy water was quite insufficient to support larvae in the numbers allotted to the dishes. The Farex made good the deficiency, and was necessary in such quantities that it became the main rather than the supplementary food. As already stressed, it was found necessary to maintain the supply at the highest possible level short of pollution, for any shortage was immediately followed by a marked retardation in growth.

Apart from their nutritional requirements, satisfactory development of the larvae was dependent primarily on the temperature of the water. They thrived at temperatures from 32–35°C., but were killed rapidly at temperatures over 37°C., although in the field healthy larvae have been collected in water heated by the sun to over 40°C. for some hours each day. At temperatures maintained below 23°C., the period

of development was protracted, and some larvae failed to pupate, or the pupae were weak and incapable of casting the larval skins. Although larvae are found in nature almost exclusively in strongly sunlit situations, nevertheless, in the laboratory, it was found that they could be reared efficiently in semi-darkness. It would seem, therefore, that direct sunlight was not essential, but was indirectly beneficial, in that it raised the temperature of the water for part of the day. In general, however, the temperature of the water was approximately the same as that of the atmosphere (fig. 2). Although this gave satisfactory results, an improved technique would include a system of heating the water to between 32°C. and 35°C. for at least part of the day.

Under the system of colony management employed, larval development was remarkably regular. Table I and fig. 5 record observations made on 5 batches of larvae. They were counted when three days old, and each batch comprised 600 larvae evenly distributed between two circular glass dishes. The various batches were set up at different times during December, 1945, and the pupae recovered from day to day were counted and sexed. The numbers of pupae formed ranged from 544 to 577, the average being 558, i.e. 93 per cent. of the larvae pupated. It will be seen from the table that the larvae pupating first were mostly males, while the last to pupate were mostly females, indicating that the period of development of male larvae was slightly shorter than that required by females. The proportion of male to female pupae finally recovered was 1 : 1.
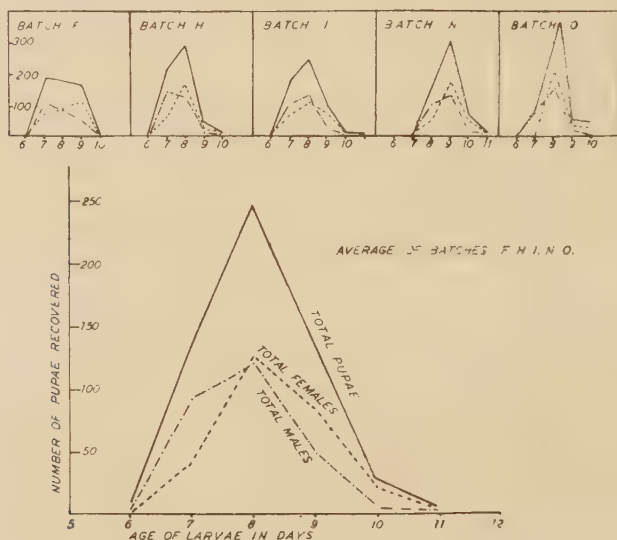
Fig. 5.—Numbers of male and female pupae recovered per day in five batches of larvae.

Pupation and emergence.

Pupation occurred mainly in the afternoons between 2 and 5 p.m. It usually began in each batch when the larvae were six or seven days old (Table I), although pupae have been recovered from 5-day old larvae, and, under adverse conditions, the commencement of pupation was sometimes postponed until the larvae were ten days old or older. There was no difference in the rate of development of male and female pupae, and both sexes began to emerge at approximately 6 p.m., the majority emerging during the night about 30–40 hours after pupation.

(245)

TABLE I.

Recovery of pupae from five batches of 600 larvae.

Age of batch days	Batch F		Batch H		Batch I		Batch N		Batch O		Average of 5 batches		
	Total males	Total females	Total males	Total females	Total males	Total females	Total males	Total females	Total males	Total females	Total pupae	Total males	Total females
6	7	1	3	1	16	3	—	—	2	—	7	6 86%	1 14%
7	113	74	145	64	104	70	18	1	87	7	136	93 68%	43 32%
8	85	95	122	173	136	109	101	54	160	203 ·	248	121 49%	127 51%
9	52	114	15	27	28	75	135	173	23	30	134	50 37%	84 63%
10	5	13	2	4	2	5	19	56	7	25	28	7 25%	21 75%
11	—	—	—	—	1	4	5	15	—	—	5	1 20%	4 80%
Total pupae re-covered	262 47%	297 53%	287 52%	269 48%	287 52%	266 48%	278 48%	299 52%	279 51%	265 49%	558	278 50%	280 50%

Larvae were 3 days old and 2nd instar at time of counting. Each batch was divided into two lots of 300 larvae. The five batches were set up on five different days.

C2

The fact that male larvae tended to pupate first was used to divide the pupae into two separate series for emergence. The first-formed pupae of each batch were returned to the breeding cages, where an excess of males was useful in ensuring fertilisation of the females. Later pupae provided the stock for the infection cages, in which, of course, only females were required. The breeding series alone is discussed here, the behaviour of those used for the transmission of malaria being recorded in another paper (Mackerras & Roberts, 1947).

Feeding behaviour of adults.

Adult activity was normally confined to the hours of darkness. Throughout the day, both sexes rested hanging from the strip of moist blanket and the sides of the cage. Towards evening, when it was just light enough to make observations, they became intensely active and a characteristic humming noise could be heard from the cages.

Newly emerged adults fed on apple juice on the first night after emergence. A few females sought blood on the following night, but the majority did not take a blood meal until 48 hours after emergence. They bit much more readily in the dark than in either daylight or electric light, but could be induced to take a blood-meal in daylight, or at night under an electric light, if they had been starved of blood for at least 24 hours beforehand. When given frequent opportunities to take a blood-meal throughout the 24 hours, their biting activity was restricted very markedly to the hours of darkness. In a test of biting activity over a period of 48 hours, an arm was inserted at hourly intervals into a cage containing 3,000 two-day-old males and females. A few females fed at 7 p.m., and subsequently between 50 and 100 fed every hour, until activity ceased at daylight on the following morning. There was no activity throughout the day, but at 7 p.m. biting started again and continued throughout the night. On the second night many females took a second blood-meal. Although the arm was left in the cage for a quarter of an hour at each period, the majority of females finished feeding within the first five minutes. These findings closely parallel the field observations of Roberts and O'Sullivan (1948), Mackerras and Aberdeen (1946), and others on the feeding behaviour of wild adults in New Guinea.

Mating.

Mating of this race was accomplished while the insects were in flight during the period of general twilight activity. In the large cages, the males did not form a compact swarm during mating, but rather flew very quickly at random round the roof of the cage. Coupled pairs were most often seen when, by contact in mid-air, they lost their flying equilibrium and dropped suddenly to the floor. If the union was complete when they touched the floor, the pair remained resting quietly attached end to end for 1 or 2 minutes, and then broke apart. Very often the union was not achieved before they reached the floor of the cage, and there was a brief struggle, resulting in the escape of the female. It is believed that union must be completed in mid-air, and that the success attained in establishing the colony by using large cages was partly due to the extra height, which allowed the insects a sufficiently long period after contact in the air before touching the floor.

Mating could be induced at any time during the night by directing an electric torch into the cage. The insects became excited, the characteristic sound of their activity increased, and, after a few minutes, coupled pairs were seen dropping to the floor of the cage. Blue light, which has been found useful in inducing mating in several other species, was not necessary with *punctulatus*.

Having mated, the females would lay several batches of fertile eggs without requiring further supplies of spermatozoa, but the fertility of some that were isolated from males eventually fell off. In the routine management of the colony,

fertilisation of all the females was assured by maintaining a preponderance of males in the breeding cages. As indicated earlier, this was accomplished by using pupae from the youngest larvae, which always produced a high proportion of males (see Table I).

Maturation of ova.

After their first blood-meal, the females required 72 hours to mature eggs. Subsequently, eggs were laid 48 hours after each blood-meal. In the breeding cages, females which had taken a blood-meal 48 hours after emergence would lay fertile eggs on the fifth night after emergence. Occasionally a few eggs were laid on the fourth night. When given an opportunity to take blood every night, some females would take two blood-meals while developing a single batch of eggs. If the blood supply was withheld from females which had been laying eggs for some time, a normal crop of eggs was obtained two nights after the final blood-meal, and then oviposition ceased almost completely, although a few eggs might be laid three nights after the final blood-meal.

The number of eggs developed by each female after the first blood-meal was low, varying from 30 to 100, compared with the 200 to 250 per batch after subsequent blood-meals.

Oviposition.

Oviposition began soon after twilight, and continued for several hours. Usually, most eggs were laid between 7 p.m. and 10 p.m., although a few might be laid at any hour of the night until dawn, when all activity ceased.

Females of *punctulatus* on the ovipositing flight hovered about six inches above the water surface. They were easily disturbed, and ceased to oviposit in a strong direct light, but could be observed in an indirect light of low intensity. One or many could be seen at a time, fluttering to and fro and occasionally dipping down just to touch the surface. The eggs were scattered at random over the surface, and were seldom seen in cohering groups of more than two or three. It is difficult to see exactly what happens, but observations strongly suggest that the eggs are normally dropped from a height. Contact with water is certainly not necessary, because experiments described below showed that females dropped their eggs even on dry paper.

This method of oviposition was described by Bates (1940) for *A. maculipennis labranchiae* (and to a lesser degree for other species), and by Russell and Rao (1942) for *A. culicifacies*. The latter authors concluded that the disappearance of *A. culicifacies* from rice fields, after the plants had reached a certain height, could be explained by the fact that the rice plants offered an obstruction to the insects during their ovipositing dance and prevented the eggs from being laid. A similar assumption may be made with regard to *punctulatus* and *farauti*, since, in the field, breeding of the former is restricted to open pools unobstructed by vegetation, whereas larvae of the latter are frequently found in pools overhung by vegetation. Although oviposition of *farauti* has not been observed, eggs have been obtained on a few occasions from caged gravid females, and invariably they were laid on the water in large cohering groups, suggesting that this subspecies may rest on the water while ovipositing. Bates found, however, that such factors as size of cage influenced the degree to which eggs were scattered in some species, so further work would be necessary before the differences between the two subspecies of *A. punctulatus* could be regarded as established.

Some observations were also made on oviposition preferences. Females of *punctulatus* preferred water containing soil to clean water in a white dish, or to clean water in a dish painted black. They would oviposit in clean water when no muddy water was available, and then did not discriminate between clean water in a white

dish and clean water in a black dish. When, however, a petri dish lined with dry, white filter paper was floated on the surface of a dish containing soil and water, eggs were laid in the surrounding water but not at all in the petri dish. When the same petri dish was lined with dry, black paper, the eggs were scattered at random over the petri dish as well as on the water. Apparently the insects could select water containing soil, and could detect white but not black objects floating on the surface of muddy water. It was apparent also that, although they sometimes performed flights similar to an oviposition flight over the white painted floor of the cage, egg laying was restricted to the dish of soil and water. It is possible, however, that, when soil and water is replaced by clean water, eggs may be laid at random on the floor of the cage as well as on the water. They have been found scattered on damp filter paper laid on the floor of a cage in which no oviposition dish was placed.

Longevity of adults in the breeding cages.

Observations on the longevity of adults in the breeding cages were made by placing a known number of pupae in the cage and determining the number of adults emerging into the cage. As many as could be found of the adults which died were removed each day, sexed, and counted, and the percentage of adults surviving was calculated.
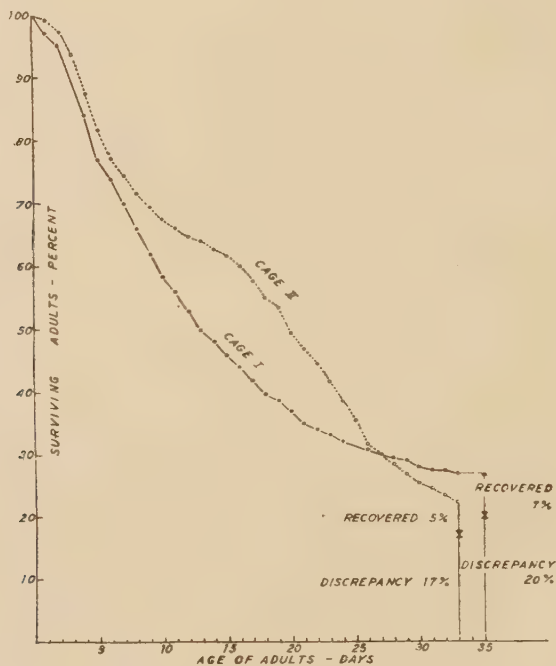
Fig. 6.—Survival curves for two breeding cages.
Cage I.   Set up with 3,000 pupae on 17.xii.1945.
Cage II.   Set up with 1,000 pupae on 23.xii.1945.

In fig. 6 the percentage of adults surviving each day is plotted for two breeding cages. It will be seen that, when the cages were cleaned out, there was a consider-able discrepancy between the calculated number and the actual number of adults recovered. This was always observed, and was due to dead mosquitos falling into crevices in the breeding dishes, in food, and between the glass sides and the wooden

frame. In spite of great care, a few bodies were missed from time to time, and in the long run formed quite a considerable proportion of the whole. However, as this loss was going on all the time it is assumed that it would not alter the shape of the curve, which tends to the sigmoid form usual in survival curves. The slightly better survival in Cage II over the middle range of its life may have been due to the fact that it contained fewer mosquitos, 1,000 as against 3,000 in Cage I. However, there was no significant difference in the percentage recovered on the 33rd day.

In fig. 7 the percentage of males and females recovered from another batch have been plotted. It will be seen that there was little difference in their survival for the first two weeks ; thereafter the females survived better, 17 per cent. of the original number of females and only 4 per cent. of the original number of males being alive on the 23rd day. This test was run during hot weather, when survival rates in all batches were relatively poor.
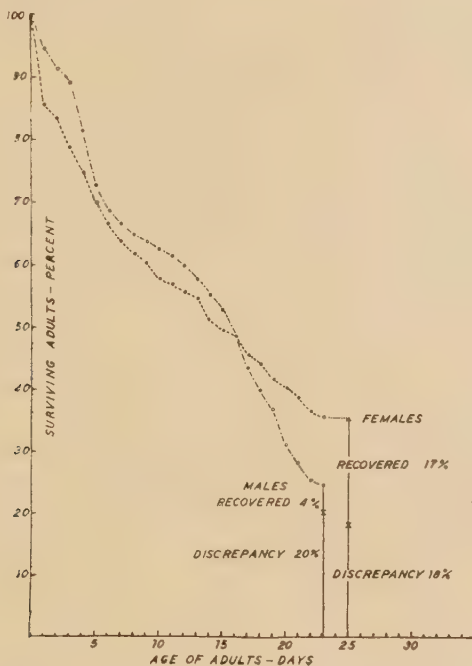
Fig. 7.—Survival curves for males and females.
Cage IV.   Set up on 7.i.1946, with 539 males and 261 females, sexed as pupae.

Discussion.

Although associated with man and apparently having a preference for human blood (Heydon, unpublished), *punctulatus* is far from being domesticated and, in the field, gives one the impression of being rather a shy and delicate insect. The work was, therefore, begun without any great optimism. This race was, however, already known to behave well in the laboratory in two important respects, namely, in the readiness with which it would take blood and in its longevity in cages (Mackerras and Roberts, 1947) ; once a few basic requirements were met, the cage colonies became established with surprising ease.

Our experience with other species is small. *A. annulipes*, Wlk., was tried in the large cages, using the standard technique. It neither fed nor lived well, showed no

sign of mating, and laid no eggs.   However, we had not at that time heard of the use of blue light to induce mating (Hackett & Bates, 1938) and tried no alternative procedures, so it cannot be suggested whether this species is truly eurygamic, or whether *punctulatus* really differs much from other Australasian Anophelines in the ease with which it can be cultivated.   *A. punctulatus farauti*, Lav., was tried in small cages 9 inches in height and some mating evidently occurred, for a small number of fertile eggs was obtained.   These, however, were too few to establish a colony.

There were several points of interest in the observations on the life cycle of *A. punctulatus*.   The duration of the various stages, under the conditions in the breeding room, were normally : egg, 42 hours ; larval, 5 to 8 days ; pupal, 30 to 40 hours ; emergence to first blood-meal 2 days ; first blood-meal to oviposition 3 days ; total from egg to egg 13 days.   These periods might be shortened somewhat at higher temperatures, though it would appear that 37°C. is approaching the upper limit of tolerance.   Eggs dried in the laboratory may hatch 2 hours after being placed in water ;   should the same happen in nature, adults could be expected $6\frac{1}{2}$ days after pools had been re-filled by rain.

A feature of the breeding cycle was the regularity with which the various processes occurred each day.   Oviposition took place early in the evening, the eggs started to hatch at varying times during the afternoon, and pupation was confined to a few hours during the afternoon.   Adult emergence began late in the afternoon, and continued throughout the night.   Biting activity was usually restricted to the hours of darkness, and mating occurred at twilight.   This diurnal rhythm of activity appears to be dependent on daily fluctuations in temperature, humidity and light intensity.   It is considered important, therefore, in the development of a satisfactory breeding technique, to establish environmental conditions of temperature, humidity and light intensity, which not only approximate to the optimum required by the species, but also fluctuate in a manner corresponding to night and day.

Finally, although it is by no means universal in Anophelines, fairly close correlation between the preferences and requirements of the different stages was indicated for this race.   Thus, mud in the breeding dishes not only proved to be the preferred oviposition site, but was definitely favourable both to eggs and larvae, which fits in well with field observations.   The method, indeed, approximates closely to the " mud-rainwater-breadcrumb " technique of Hackett and Bates (1938), and may have a wide application ;   it seems to be peculiarly suitable for *punctulatus*.

Summary.

The room used for housing a colony of *Anopheles punctulatus punctulatus* is described.   Its main features were an insulation system to enable some degree of temperature control during the winter months, and eight windows to ensure that the room had good natural lighting.

The adults were kept in breeding cages, which were large enough to allow the insects to manoeuvre during mating and oviposition, and designed to ensure a high humidity and at the same time expose the adults to daily fluctuations in light intensity.   Provision was made for easy access to the interior during feeding and management operations.

The essential feature of larval management was that the larvae were kept throughout their development as separate batches, representing daily age groups.   During the first three days after hatching they were left in the dishes containing soil and water in which the eggs were laid.   From the third day, they were reared in shallow glass dishes.   They were fed with Farex at intervals throughout the day.

Pupae were washed and bulked together in small enamel dishes at the rate of 150 per square inch. The first pupae formed in each batch contained a high proportion of males, and were returned to the breeding cages. Those formed later contained a high proportion of females, and were placed in small cages, in which the adults were kept for infection with malaria.

A diurnal rhythm of adult activity was observed. Biting normally occurred at night ; mating took place at twilight, and was accomplished while the insects were in flight.

Eggs matured in 72 hours after the first blood-meal, and 48 hours after subsequent blood-meals. Oviposition began soon after twilight, the eggs being scattered while the insects performed a hovering dance over the water surface ; water containing soil was preferred as an oviposition site. Eggs may withstand drying for at least $5\frac{1}{2}$ days.

Developmental periods observed were : egg, 42 hours ; larval, 5–8 days ; pupal, 30–40 hours. Factors favouring larval development were shallow water, muddy or sandy substrate, liberal food, and water temperature between 30 and 35°C. The 50 per cent. survival period for adults in the breeding cages was about 15 days.

Acknowledgements.

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GLOSSINA PALLIDIPES AND OPEN COUNTRY IN THE COASTAL AREA OF KENYA.

By J. Y. MOGGRIDGE.

Tsetse Research Department, Tanganyika Territory.

In the course of a general investigation of the tsetses of the coastal area of Kenya in the neighbourhood of Kilifi (some 40 miles north of Mombasa), a number of observations were made on the extent to which *Glossina pallidipes*, Aust., would enter and cross open country, both natural and artificial. This short account is offered since the experiments have a bearing on the use of barrier clearings against this tsetse and the extent to which cattle may approach infested bush with impunity.

Crossing Open Ground.

Experiments were undertaken to ascertain the distance at which a target moving in the open will attract tsetses from a thicket. The site selected was very suitable for the purpose as the thicket edge was sharply defined and quite straight, and large populations of *G. pallidipes* and *G. austeni*, Newst., were known to be present in it. *G. brevipalpis*, Newst., were present also. The area in which the transects were set out was an artificial clearing, absolutely open and covered with grass to knee height. The transects were 350 yards long and laid out parallel to the edge of the thicket at distances of 25, 50, 100 and 125 yards. The catching party consisted of four with two screens and a recorder who read the instruments and wrote down the data. The method was that described for similar experiments with *G. swynnertoni*, Aust., at Shinyanga (Moggridge, 1936a). The party patrolled each transect twice, moving slowly from one end to the other and then back again. The transect most remote from the bush edge was traversed first and the others in succession. Thirteen experiments were carried out in October and November 1935, six in the late evening and seven in the early morning, the average time in each case being 54 minutes. *G. pallidipes* was the only species of tsetse captured. The catches for the different transects, in 13 experiments during the dry season of 1935, were very small indeed as will be seen from the table below.

Total number of *G. pallidipes* captured :—

Distance of transect from thicket edge	Total number captured
25 yards	52
50 yards	13
100 yards	7
Up to 100 yards	72
125 yards	2

These results were obtained during dry season conditions with temperatures ranging between 20·8°C. and 31·4°C. and saturation deficits between 8 and 14 mb.

During 1938–39 an extensive landing ground was made in dense thicket growing on " coral rag " rock. The landing ground was 1,400 yards long and 600 yards broad. The surface of the ground was practically flat and the grass was kept short on the run-way. The edges of the thicket surrounding the landing ground were cleanly cut, and it was known to harbour large populations of all three species of tsetse on the north side.

It was decided to supplement the series of experiments mentioned above by others carried out on this landing ground in the wet season with oxen as targets. The procedure was similar to that used on the earlier occasion but 4 to 5 oxen took the place of screens. The transects were placed at distances of 80, 180, 230 and 280 yards from the north thicket edge and ran parallel with it. The transects at their east and west extremities were not less than 400 yards from the thicket. Each transect was 400 yards long. From 25th May to 27th July, 1939, 15 experiments were carried out, each lasting, on the average, 53 minutes and starting sometimes at 7 a.m. and sometimes at 8 a.m. Conditions throughout were excellently suited to tsetse activity. Just within the thicket and parallel to the transects, but at some distance to the east of them, a control catch over 400 yards was made simultaneously with each experimental transect catch. During these catches *G. pallidipes* were taken at the rate of 87 to the hour and *G. austeni* at the rate of 22 to the hour ; no *G. austeni* were taken in the open, but one male *G. brevipalpis* was captured on the 80 yard transect.

Total number of *G. pallidipes* captured in 15 experiments during the wet season of 1939 :—

Distance of transect from thicket edge	Total numbers captured
80 yards	35 (20 males and 15 females)
180 yards	12 (8 males and 4 females)
230 yards	10 (7 males and 3 females)
280 yards	2 (2 males)

These experiments support the conclusion that under coastal conditions these tsetses do not venture far into the open even under the cool and humid conditions that prevail in the mornings during the wet season, but that they do so to a somewhat greater distance than in the dry season, though in smaller numbers.

Carriage from Thicket on Man and Animals.

During May, June and July, 1939, a number of experiments were undertaken to find out if tsetses would follow natives into the open or be carried by them from the thicket edge.

Five parallel paths were cut for a distance of 200 yards into the " coral rag " thicket of The Transect Experiment Control described in the previous section and the following experiments were carried out at about 8 a.m.

On men alone.

In two of the seven experiments in this series the African assistants wore green canvas rain capes. Four African assistants and the writer entered the thicket, each on his own path, and returned by the same path to the thicket edge. Five *G. pallidipes*, one *G. austeni* and one *G. brevipalpis* were carried from the thicket edge on to the landing ground. No attempt was made to interfere with the tsetses which flew away, usually gorged, at distances not greater than 150 yards from the thicket edge.

On men carrying loads.

Four experiments with four African assistants, wearing rain capes and carrying loads of firewood, were supervised by the writer. On the first occasion two female *G. pallidipes* were carried on the legs of natives, and these flew off gorged within 100 yards of the thicket edge. Subsequent experiments produced negative results. On three other occasions large bundles of grass were substituted for the firewood but again with no result.

On men and oxen.

Four experiments were carried out on similar lines to those described above but the assistants were without loads and each pair was accompanied by an ox. Three *G. pallidipes* were carried from the thicket with the cattle as far as 200 yards in the open during this series of experiments.

The temperatures ranged from 22·2° to 27·2°C. and saturation deficit from 0·5 to 7·2 mb. whilst the wind was blowing diagonally from the transects to the bush at an average velocity of 4·6 m.p.h.

Glade Experiments.

Previous observations and experiments had tended to show that *G. pallidipes* would not readily leave the thicket edge to attack the catching party. On a fly round made from May to November 1936, sections were put in with the object of securing further data. Section A passed close along the edge of a large thicket clump, section B doubled back and passed right through the middle of this thicket clump, while section C passed in the same direction as section A on the other side of the same thicket clump and between it and the main thicket. (See fig. 1.) The
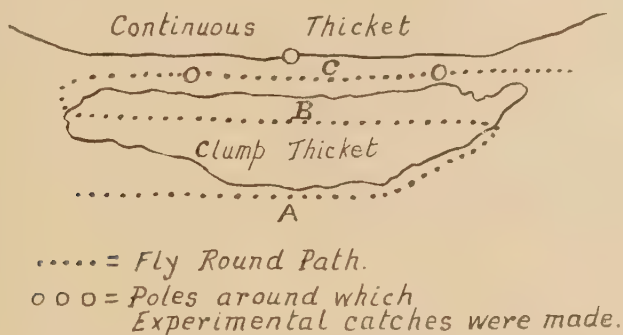
Fig. 1.—Diagram to illustrate Glade experiments.

width of the glade between this clump and the thicket proper was only 16 yards. The fly round was made in the morning at about 8.30 a.m., and in the afternoon at about 4.30 p.m. ; the sections were all about the same length—a little over 200 yards. The mean catches per 500 yards of *G. pallidipes* for the three sections over 20 fly round catches made during the humid season of 1936 are given below :—

Section A		Section B		Section C	
Tsetses	Female per cent.	Tsetses	Female per cent.	Tsetses	Female per cent.
11	41·3	27	28·9	4	58·2

It will be seen that, at a distance of only 8 yards from two heavily infested thicket areas, an average of only 4 tsetses per 500 yards was captured, yet when the party walked through the clump thicket the catch was raised to 27 tsetses per 500 yards. The low percentage of female tsetses which attacked within the thicket is in interesting contrast to the high percentages captured in the open between the two thickets and along the edge of the one thicket.

In order to find out more about this apparent reluctance to attack across the open, even though the distance was very small, a series of catches were made in the glade between the two thicket areas on section C. Three catches were made in the early mornings during July and August, 1936 and one in December, 1937. Two bait cattle and four catchers were used.

A line was marked in the grass exactly mid-way between the thickets and at each end of the glade a pole was erected. At each pole a catch was made over a number of minutes and during this time the party moved around in a small circle about the pole. The party then moved down the glade along the central line and another catch was made at the other pole. The distance between the poles was 150 yards. (See fig. 1.) The behaviour of the tsetses on the arrival of the party appeared to be largely one of curiosity for they simply settled on the grass close by and made no move to attack. After the catches had been made in the glade, catches were made in the thicket along section B. In July and August *G. pallidipes* were taken in the glade at the rate of 50 per hour over average periods exceeding $2\frac{1}{2}$ hours ; the rate of catching in December was very much higher but the period over which the catches were made was short (6 minutes). These experiments do not confirm the data collected on fly rounds recorded earlier ; those in July and August were carried out while the fly round was being made as a routine task and neither the hunger judged by external appearance, nor the female percentage, differs from the values obtained on the round itself. There is, therefore, no reason to suppose that any physical factor entered into the experimental catches that was not present at the time of the fly rounds over the identical ground. It was clear that the tsetses were not at all hungry and hunger may, therefore, be ruled out as a principal reason for attack. The main difference between the fly round catches and the stationary catches was in the time the party remained in the glade and the impression was gained that the tsetses were attracted only by the repeated movement in the glade. The one short passage along the glade made on the fly round did not exert on the tsetses sufficient stimulus to cause them to cross the intervening open space.

Discussion.

These experiments show that during the dry season on the Kenya Coast *G. pallidipes* in small numbers will range out into the open to attack as much as 100 yards from the thicket, but that beyond that distance only very few attack. In the wet season, fewer still venture out, but those that do will attack up to 230 yards ; beyond this range only very few attack. Similar experiments with *G. swynnertoni* (Moggridge, 1936a) showed a very similar result, for under dry season conditions in Shinyanga (Tanganyika Territory) when the grass was long, this species failed to attack at distances greater than 100 yards, although later in the dry season, over ground which had been partially burnt so that conditions gave excellent visibility, a few did attack up to 300 yards.

An attempt at control showed that a cleared barrier of 140 yards (with considerable numbers of trees left standing) did not prevent *G. pallidipes* from crossing in appreciable numbers into the experimental area ; this result would be expected in light of the above experiments.

G. pallidipes seems, on the Kenya Coast, to be carried into open country by men and oxen to a far less extent than *G. swynnertoni* in Shinyanga, where this species was found to be carried over 1,000 yards in the dry season, and up to 400 yards in the wet season (though in greater numbers). During the wet season *G. pallidipes* was not carried beyond 150 yards on man, though they stayed with cattle for a distance of some 200 yards. It is, however, likely that faster moving parties, such as game moving rapidly across a narrow clearing, or lorries crossing the clearing, would take even this species very much further.

It is interesting to compare these results at Kilifi in which *G. pallidipes* were not carried to any extent from their natural habitat on loads carried by natives with the observations and deductions made by the author in Italian Somaliland (Moggridge, 1936b). The author encountered a number of native women in Italian Somaliland carrying loads of fire wood under which were 4 *pallidipes*. The temperature and humidity at the time of these observations were very similar to those recorded during the Kilifi experiments.

The contrasting data obtained from the fly rounds and the glade experiments, besides revealing that a persisting target is attractive to *G. pallidipes* (at least over very short distances from the habitat), show that fly round data cannot safely be used to predict tsetse behaviour under circumstances other than those pertaining on fly rounds. For example, an interpretation of the fly round data to mean that stock may safely be grazed up to within a few yards of *pallidipes* infested bush would be dangerous.

Summary.

Glossina pallidipes does not readily attack objects moving along the edge of vegetation forming its habitat ; fewer tsetse attack under humid than under dry conditions, but those that do seem to range further.

Parties of men moving out of heavily infested thicket did not carry tsetse far into the open. Few tsetses attached themselves to the men and these became gorged within a few yards of the thicket edge. Similar results were obtained when bait cattle were used.

Experimental catches in a glade showed, even during the humid season, an extraordinary reluctance on the part of *G. pallidipes* to approach a party moving through the glade even at so short a distance as 8 yards from the thicket, but further experiments showed that a party staying in the glade for a short time made a much larger catch.

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A NEW VARIETY OF *ANOPHELES AITKENI* FROM BORNEO.

By John McArthur, M.R.C.S., L.R.C.P.
*Malaria Research Officer, North Borneo.**

The following is a description of a mosquito found to occur in the hills surrounding Tambunan Plain, North Borneo, whilst working with the Malaria Research Department, in 1939–42. It appears to represent a new variety of the *Anopheles aitkeni* type, which it is proposed to name *Anopheles aitkeni borneensis*.

Anopheles aitkeni var. borneensis, nov.

Adult Male small, dark, unornamented, and inconspicuous; without markings, and assuming a very culex-like attitude when at rest. It is indistinguishable externally from local specimens of *A. aitkeni bengalensis*, and from the published description of the type form by Gater (1935), but differs from both in the anatomy of the male terminalia, and in the tendency to a slightly greater length of the proboscis.

Scales on vertex, thin, narrow, with bifurcate tips. Frontal tuft short, with few setae. Labium long, thin, and unornamented (may be a little longer than the palpi). Antennae without scales. Palpi narrow, expanded, with few setae, unornamented and pale greenish. Mesonotum uniformly brown. Pleura pale greenish without scales. Anterior pronotum without scales. Propleural setae usually single. Spiracular setae usually absent. Upper mesepimeral setae usually present. Wings dark, unornamented. Anterior forked cell (Cell R_2) nearly twice as long as posterior forked cell (Cell M_1). Legs dark, long, thin, unornamented. Hind leg with sometimes a pale area on tibio-tarsal joint. Coxae pale. Coxae and trochanters without scales. Abdomen dark, lighter ventrally, greenish, without scales.

Male terminalia of the *Anopheles aitkeni* type. The ninth tergite without processes. The coxites are without scales, and the internal spine is near the apex. The two parabasal spines are about equal in thickness, the outer being longer ; both are curved inwards at the tip. The phallosome is short and smooth, without leaflets or thickening of the apex, and half the length of the coxite.

Nomenclature of the Harpago.

There is some confusion in the nomenclature of the male terminalia of *Anopheles aitkeni*, especially of the harpago, the lobes of which have different, and in some cases contradictory, names according to different authors.

It is considered desirable, in order to prevent confusion, to refer to each of the four generally recognised parts of the harpago of *A. aitkeni* simply as parts A, B, C, and D, in that order from above (in the morphological sense) downwards and inwards.

These parts have been named by different authors as follows :—

A. Ventral lobe (Puri 1930)
   Dorsal and external part of dorsal lobe (Christophers 1933)
   Upper portion of upper lobe (Gater 1935)

B. Middle lobe (Puri 1930)
   Ventral and internal portion of dorsal lobe (Christophers 1933)
   Lower portion of upper lobe (Gater 1935)

C. Part of dorsal lobe (Puri 1930)
   Outer part of ventral membranous portion (Christophers 1933)
   Middle lobe (Gater 1935)

D

D. Part of dorsal lobe (Puri 1930)

 Inner part of ventral portion (Christophers 1933)
 Ventral lobe (Gater 1935)

It will thus be seen that the designation of the four parts by letters is not without justification for the purpose of comparing the harpago of the new variety with those already described.

Harpago (fig. 1).

On this basis, the harpago of the new variety is divided into four parts, A, B, C, and D. A is dorsal (in the morphological sense) and external ; B is internal and ventral to A ; C is internal—but rather dorsal—to B ; and D is the most ventral and internal, and fused with its fellow of the opposite side. Parts A and B are strongly chitinised ; half of C is chitinised, and the other half of C and D are membranous.

Part A is provided with two blade-like setae, the outer (and longer) of which shows traces of division into two at its base. Part B is provided with one long stout blade-like seta, showing traces of division into two toward its base. The setae of Parts A and B do not tend to form a club-like handle, as in the other varieties. Part C, which stands out dorsally, although internal to Part B, is provided with two long setae, both recurved, the inner and posterior being longer than the outer and anterior. Part C is chitinised around the base of the latter. Part D is membranous and provided with one long recurved seta. In addition it is well covered with short hairs, extending to Part C.

On the basis of this nomenclature, the differences between the varieties may be tabulated as follows :—

	A	B	C	D
A. aitkeni	Three blade-like (in a club-like bundle)	Two expanded	Two blade-like	One blade-like (and small accessory)
A. a. bengalensis ...	Two blade-like (all rather shorter)	Two blade-like	Two blade-like	One blade-like
A. a. palmatus ...	Two blade-like	Two blade-like (partly fused)	One with tip expanded	Three stout, with accessory hairs
A. a. borneensis ...	Two blade-like (not in club-like bundle)	One blade-like	Two blade-like	One blade-like
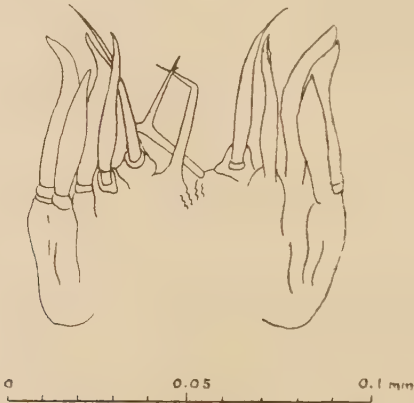
Fig. 1.—Harpago of *Anopheles aitkeni borneensis*, var. n.

LARVA very black in colour to the naked eye, compared with the pale brown of *A. aitkeni bengalensis* with which it is often associated. There are innumerable small hairs all over the body and its anatomical features are as follows :—

Inner anterior clypeal hairs are placed about the same distance apart as the inner and outer hairs on one side. They have about twelve side branches (8–14) distributed along the length of the shaft, but mainly distally. Outer anterior clypeal hairs are about one-third the length of the inner, with about ten branches (9–12), mainly from the base. Posterior clypeal hairs are slightly shorter than the outer anterior, with about four to nine branches. Sutural hairs are about the length of the posterior clypeal hairs with about two branches (1–3). Trans-sutural hairs, are about the length of the posterior clypeal, with about five branches (3–7). Antennal hairs arise on the dorso-internal surface ; they are almost half the length of the shaft, and have about 7–15 branches.

The inner submedian prothoracic hairs have about 10–13 branches, the central about 11–19 branches, with a large root. Metathoracic palmate hairs have about 13–19 narrow lanceolate leaflets.

Pleural hairs : Prothoracic.—2 long, simple ; 1 long, branched (3–7 branches) ; 1 short branched (3–7 branches). Mesothoracic.—1 long, simple (or maybe two branches) ; 1 long, branched (2–3 branches) ; 1 short, simple or branched (1–3 branches) ; sometimes one minute, simple or branched (1–3). Metathoracic.—1 long, simple ; 1 long, branched (2–4 branches), 1 short branched (2–4 branches) ; sometimes one minute, simple.

Abdominal palmate hairs : No. I is rudimentary ; Nos. II–VII are fully developed with about twenty leaflets.

Differences from other varieties of *Anopheles aitkeni* are chiefly in the anterior clypeal hairs and these are summarised below and illustrated in fig. 2.

		Inner	Outer
A. aitkeni	...	Two branches	Two branches
A. a. bengalensis	...	Four branches	Three branches
A. a. palmatus	...	Simple	Simple or bifid
A. a. borneensis	...	8–14 branches	9–12 branches

A. aitkeni borneensis shows certain differences from *A. aitkeni*, James, *A. aitkeni bengalensis*, Puri, and *A. aitkeni palmatus*, Rodenwaldt, which are all found in the same neighbourhood. It is apparently indistinguishable from *A. aitkeni* in the adult stage except by the male terminalia and possibly by the greater length of the proboscis. The larva shows considerable differences from the known varieties, especially in the multiple branching of the clypeal hairs (about 12 in the case of the inner, and about 10 in the case of the outer), as well as in other less prominent features.

Type Material.

The material used as a basis for the above description consisted of larvae and of adults hatched out in the laboratory from mature larvae, together with their larval and pupal pelts.

The larvae for both mounting and hatching were all taken in the hills above Kampong Naudu, Tambunan Plain, North Borneo, in clear running water under dense jungle shade, on 16th December 1941—two weeks before the Japanese occupied the area—by Mr. Francis Yong.

Unfortunately nearly all the specimens have now been destroyed. Those in Borneo were preserved during the first 18 months of the Japanese occupation, but

were finally lost during my internment. The three males and three females used as a basis for the above description, one pupa and twelve larvae all perished in the destruction of Jesselton in 1945, although the preparation of male terminalia was recovered from the ruins. Earlier specimens were sent to the Institute for Medical Research, Kuala Lumpur, on 4th July 1941, and one adult male and four larvae were sent to the Malaria Institute of India on 30th September 1941. Four other preparations of larvae have been recovered from the ruins of Jesselton, and these, together with the preparation of the male terminalia, are preserved in the Malaria Research Headquarters in North Borneo.
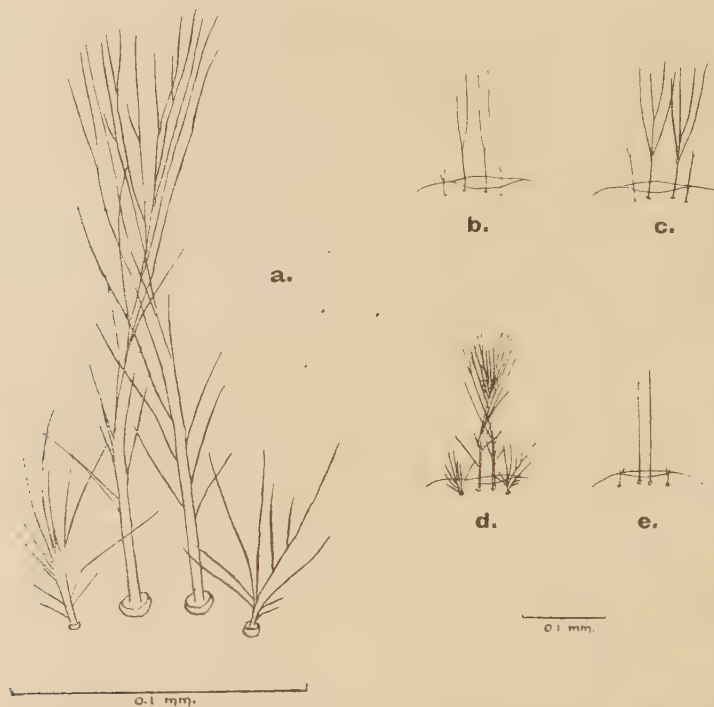
Fig. 2.—Anterior clypeal hairs: a, d, var. *borneensis*; b, typical *aitkeni*; c, var. *bengalensis*; e, *palmatus*.

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THE *ANOPHELES* OF TAMBUNAN, NORTH BORNEO.

By John McArthur, M.R.C.S., L.R.C.P.

*Malaria Research Officer, North Borneo.**

Tambunan is an elevated and isolated plain in the interior of North Borneo, occupied by irrigated paddy cultivation, surrounded by jungle-covered hills, and inhabited by a primitive native population subject to severe malaria.

The Malaria Research Department of North Borneo carried out surveys in this locality during 1939–42 for larval and adult *Anopheles* as part of an investigation into the causes and control of malaria in Borneo. The main findings of this work, and the incrimination of *A. leucosphyrus*, Dön., as a vector of malaria, have been described elsewhere (McArthur 1947), but brief accounts are given below of the habits and characteristics of the *Anopheles* in the locality.

In addition to more extended larval surveys, monthly examinations were made over a period of two years in every breeding place within a selected square mile of territory. This square mile included typical examples of every possible type of water in the locality. There were springs and jungle seepages in the hills, and innumerable collections of water on the inhabited plain, such as irrigated paddy fields and their channels, streams, pools, swamps, puddles and buffalo hoof-prints ; and these were in every degree of pollution, and in every variety of sunlight and shade.

Larval examinations yielded nearly 48,000 larvae belonging to 12 species or varieties, in the following numbers and proportions (records of some of the smaller figures were lost during the Japanese occupation, but these were less than 0·5 per cent.) :—

Species				Total	Proportion
					Per cent.
A. barbirostris, Wulp	...	...	...	26,693	56
A. kochi, Dön.	...	...	...	8,279	17
A. maculatus, Theo.	...	...	...	7,114	15
A. philippinensis, Ludl.		...	...	5,080	11
A. karwari, James	...	...	...	271	1
A. aitkeni bengalensis, Puri		...	...	113	
A. leucosphyrus, Dön.	...	...	...	97	
A. barbumbrosus, Strickl. & Chowd.		...	79	All less
A. aitkeni borneensis, McArthur		...	59	than 0·5
A. tessellatus, Theo.	...	...	...	?	per cent.
A. aitkeni palmatus, Rodenw.	...	...	?	
A. aitkeni, James	...	...	...	?	
Total	...	...		47,785+	

Adults were searched for by house examination in the conventional way, also by human bait trappings, by jungle searching, by animal bait trapping on ponies and buffaloes, and finally by the examination of defective mosquito nets in native houses in the early mornings. House searching as practised in other countries was carried out thoroughly for three years, in hundreds of situations, but resulted in the incredibly small yield of only 10 adults.

The results of human bait trapping, although not quite so insignificant, were also astonishingly meagre. There was only a yield of 172 *Anopheles* in 1,860 operations of

the trap, over the same number of hours. Jungle searching for 46 days yielded 1,626 *Anopheles*, but almost entirely of the two most common species. Animal bait trapping was the most fruitful source of material, yielding over 10,000 specimens. Searches by natives in their defective mosquito nets in the early morning, however, although yielding only 716 specimens, proved the most valuable method ; it provided the bulk of the infected specimens, and was the means of incriminating the vector of malaria in the locality.

The numbers of each species captured by the different methods were as follows :—

Species	House Searching	Human Bait Trapping	Jungle Searching	Animal Bait Trapping	Trapping by Natives	Total
A. philippinensis ...	—	35	1,147	4,454	54	5,690
A. kochi	4	53	447	4,542	32	5,078
A. leucosphyrus ...	2	51	3	172	574	802
A. barbirostris	3	20	13	552	24	612
A. maculatus ...	—	9	11	142	25	187
A. karwari ...	—	4	5	130	6	145
A. tessellatus ...	—	—	—	20	1	21
A. barbumbrosus ...	1	—	—	—	—	1
A. ludlowi ...	—	—	—	1	—	1
TOTALS	10	172	1,626	10,013	716	12,537

Of these, *A. leucosphyrus* ultimately proved to be the only vector of the hyper-endemic malaria of the locality. At first it was found only in minute numbers by the conventional methods of search, and considered to be rare and harmless ; but when its habits were understood it was found to be not only far from rare, but a widespread and common although very elusive mosquito.

The characteristics and habits in Tambunan of all the *Anopheles* found are summarised below.

Anopheles leucosphyrus, Dönitz.

This species has never been considered to be of any great importance in other parts of the world ; in fact in Malaya it is sometimes regarded as being an indication of the absence of dangerous species. In Borneo, however, it appears to be one of the most widespread and dangerous mosquitos. It is, however, so difficult to find that it is easily missed, and may be regarded as absent from an area when in fact it is widespread and responsible for much malaria.

A. leucosphyrus was found to breed in seepages, in dense jungle shade, in the presence of dead leaves. These seepages might be at the stream-head, along the course of a stream, or at a hillfoot, provided there was jungle shade. Breeding places might be found in the hoof-prints of cattle in the jungle. It was, therefore, difficult to find as a larva because it was often necessary to hack a way into the jungle before its breeding place could be discovered. It generally bred alone, but was occasionally found in association with *A. aitkeni bengalensis*.

The larva was very shy, easily disturbed, and might remain submerged in the mud for as long as five minutes or more, so that it was often difficult to find even when its breeding place was opened up and clearly defined. It was so elusive that without a proper technique it could be missed in a district for months. In order to collect the larvae, it was necessary to know where to look for them and to be prepared to hack a way in to expose a breeding place ; it involved getting very wet and dirty, and dipping to the bottom of the seepages, and then to have sufficient patience to wait until larvae, if present, had come to the surface.

It had very little appetite for animal blood and a much greater preference for human blood, with an anthropophilic index of about 90 per cent., making it a very dangerous mosquito. It was found that it might infest an area, and yet be apparently completely absent, chiefly because its habit was to feed very late at night or in the early hours of the morning, and to fly away again before light.

The adult is very handsome, and easily distinguished from all other *Anopheles* in the locality by the fact that the tibio-tarsal joint of its hind leg was very conspicuously white, so that with the naked eye a prominent white band was seen in the middle of the leg. Adults were best obtained by searching defective mosquito nets in the early mornings, or sometimes after midnight in native huts in malarious areas near jungle ravines.

Relation to Malaria.

In spite of the difficulty of capturing either larvae or adults, *A. leucosphyrus* has been recorded more often than any other species in Borneo, so that it is probably the most widespread *Anopheles* present. It has also been more frequently found infected, and more highly infected, than any other mosquito in Borneo. For these reasons, it must be regarded as probably the most important insect in the island.

Anopheles maculatus, Theobald.

This is one of the most dangerous of Malayan *Anopheles*, and on that account has always been considered to be the chief vector of malaria in Borneo. This belief, however, has been shown to be without foundation, and it is almost certainly harmless in Borneo. It is not of such importance in other parts of the world as in Malaya, and in some places it is a non-vector. In Tambunan and elsewhere in Borneo, it is widespread in its breeding, and was at first under considerable suspicion, but there is no direct evidence for its incrimination.

A. maculatus was found to breed in clear spring water in sunlight, in springs, seepages, streams, and wells. The water was sometimes flowing fairly freely, and often had a sandy or muddy bed with stones, and grassy edges. Its breeding places were typically in cleared ravines, in which streams and seepages of clear spring water had been exposed to the sun.

This mosquito was often easy to see in water, and might be dipped off with a spoon. If it was not too great, the entire water in the seepage was scooped up, and dipping could also be carried out along the grassy banks of typical streams.

A. maculatus formed only 3 per cent. of those captured in the human bait trap. It was not often captured on cattle or ponies but, in spite of this, those taken in relation to animals represented 85 per cent. of the *A. maculatus* captured. Insufficient suitable specimens were captured to permit of adequate precipitin tests. Only one such specimen was taken resting in the jungle and this proved to have fed on cattle blood and not on man. The adult was not often seen. It was a decorated mosquito with speckled legs, and could be distinguished by the naked eye from the far more common *A. kochi* by the absence of the abdominal tufts so characteristic of this species.

Relation to Malaria.

The relation of this species to malaria in Borneo is being fully dealt with elsewhere, but its importance in Tambunan can be summarised as follows :—

It bred in fairly close association with the highest spleen rates, and this was circumstantial evidence in support of the belief that it was a vector. On the other hand, two of its most extensive breeding places were found in very close proximity to two of the healthiest areas, while in some of the most intensely malarious areas it was practically absent. This peculiar distribution was explained by the incrimination,

by dissection, of *A. leucosphyrus* as the vector, and by the assumption that *A. maculatus* was not responsible for much, if any, malaria.

The marked preference of *A. maculatus* for cattle suggested that it could not be of any importance as a vector, and its extreme rarity in houses supported this. Further, a series of 177 dissections carried out over a period of two years, on specimens obtained only with great difficulty, proved negative ; while 213 negative dissections have been carried out throughout Borneo. It was concluded, therefore, that *A. maculatus* was not a vector of malaria in Tambunan.

Anopheles barbirostris, Wulp.

This is the most widely breeding species in Tambunan Plain, representing 57 per cent. of all the larvae identified during two years routine monthly surveys. The number of adults found, however, was very small in proportion. During the early part of the paddy irrigation season it is the commonest species found in the paddy fields. It was found also breeding in pools, wells, swamps, seepages and streams. It appeared to prefer vegetation, or a certain degree of pollution in the water, and was found amongst grasses and other vegetation fringing pools in full sunlight. It bred in large numbers in the paddy fields when these were first planted, but gradually diminished with the increasing shade of the growing plants, and was soon surpassed by the number of *A. philippinensis*. When the irrigation was cut off, however, at the end of the season, the water stagnated and resulted in the sudden and complete disappearance of *A. philippinensis*, and the restoration of *A. barbirostris* to predominance.

A. barbirostris was not a shy larva, and was easily caught. It quickly rose to the surface again after submerging and, being a large larva, was readily seen. It could be taken easily by dipping round among the grasses and vegetation at the edges of pools or paddy fields.

The adult was a large, black, very shy mosquito which was rarely seen. It was taken occasionally in the human bait trap, and formed 5 per cent. of those so taken. Although still rare, it was taken much more frequently feeding on cattle. It was occasionally taken by beating jungle along the banks of cool, well-shaded streams. It was also seen on five occasions attempting to feed on members of the staff who were at work in the jungle during daylight, and was the only *Anopheles* which was ever found so doing in the locality.

Relation to Malaria.

It is generally considered to be of little importance as a vector of malaria, but occasionally it has been shown to be responsible for transmission in Malaya (Hodgkin & Johnston, 1935), and Dutch East Indies (Swellengrebel & others, 1919). In 1940, also, it was found infected in 12 per cent. of dissections in Celebes (Overbeek, personal communication), and may therefore be of some importance.

The proportion taken on cattle represented 90 per cent., while those taken in the human bait trap were only 10 per cent., of the total. The chances therefore of any single specimen being able to transmit a malaria infection by two suitable human blood meals were small. This, in conjunction with its extreme rarity in houses, was an adequate reason why this mosquito should be of little or no importance as a vector in Tambunan and this was confirmed by the fact that 521 dissections were all negative.

Anopheles kochi, Dönitz.

This species was found very commonly both as larva and adult and at most times was the most frequently captured adult mosquito in Tambunan. It bred widely with *Culex*, in pools, swamps, seepages, puddles, hoof-prints, and buffalo wallows.

Although the water might be clear, it often bred in cloudy polluted water. The water was generally still, in full sunlight, with a muddy bed, often with earthy edges and with a green, red or brown scum. It bred in paddy fields when the flow of irrigation had been cut off and the water began to stagnate.

This mosquito was very shy, and quickly dived if disturbed, but rapidly rose to the surface again. It could be taken by dipping among grasses or in pools, in the same way as, but a little quicker than, *A. barbirostris*.

A. kochi is a medium sized mosquito, decorated, and with speckled legs. It was easily identified with a lens, or even with the naked eye, and distinguished from all other local species, by the tufts of scales that are prominent on the under surface of the abdomen. The adult was by far the most common *Anopheles* captured by pony trapping, representing about 4 out of every 5 specimens taken, except during the paddy season when *A. philippinensis* also occurred in great numbers. It was also the commonest species taken in the human bait trap. In spite of this, however, the yield from the human bait trap represented only 3 per cent. of the total *A. kochi* captured, so that it had the highest preference for animal blood of all the *Anopheles*. This preference for animal blood was confirmed by a small series of precipitin tests. These were carried out on 14 specimens of females captured in the jungle, all of which proved to contain cattle blood.

Relation to Malaria.

It is not generally considered to be of any importance in the transmission of malaria, although it has been found infected in Sumatra (Doorenbos, 1931).

The slight interest of this species in human blood in Tambunan was not in keeping with its being a vector of malaria, in spite of the fact that it was the commonest species taken in the human bait trap, and this was confirmed by the fact that 541 dissections of *A. kochi* were all negative.

A. kochi was, therefore, not regarded as a vector of malaria in Tambunan, and this was supported by the fact that the distribution of the highest spleen rates corresponded to situations where *A. kochi* bred least.

Anopheles philippinensis, Ludlow.

During the irrigation season this species was found in enormous numbers, breeding over almost the entire surface of the plain, and at that time it was found to be one of the commonest mosquitos taken by pony trapping or in the human bait trap.

A. philippinensis was a typical paddy-field breeder, being found in the irrigated fields, irrigation channels and elsewhere in fresh, still, or gently flowing water, generally with grasses, paddy, and floating algae. It increased with the increasing shade of the growing paddy, until ultimately it became the predominant species, to disappear, however, immediately the flow of water was cut off and stagnation commenced. It was occasionally found in other situations, in pools of clear water with grassy edges and muddy beds, and elsewhere.

It was a rather shy larva, and was dipped for round the edges of paddy fields and at the roots of vegetation, in the same way as for *A. barbirostris* but a little more quickly.

The adult could readily be recognised in Tambunan with the naked eye by the striking white tarsi of the hind legs. It could be taken, during the irrigation season, in very considerable numbers feeding on animals, and in small numbers in the human-bait trap. As was the case with all other *Anopheles*, except *A. leucosphyrus*, it had a great preference for animal blood, being captured in association with man in only 6 per cent. of cases. This preference was confirmed by a small series of 19 precipitin tests carried out on individuals captured in the jungle. All of the 19 specimens proved to have fed on cattle blood.

Relation to Malaria.

A. *philippinensis* was occasionally seen attempting to feed on human beings, for example on the operator engaged in animal-bait trapping ; but this was probably only on account of its very large numbers and it had almost certainly no relation to malaria in Tambunan.

Dissections of 567 specimens proved negative, indicating that it could play no important part in malaria transmission ; a conclusion supported by the fact that malaria had no evident seasonal incidence, as it would have if this seasonal mosquito played an important part. Moreover, the distribution of the greatest breeding was the reverse of the distribution of the highest spleen rates.

In some parts of the world, although it has a low anthropophilic index, A. *philippinensis* is a vector of malaria of some importance simply on account of its enormous numbers. In Borneo, therefore, as was the case with other *Anopheles* having a great preference for animal blood, it remained to be proved whether it had any relation to malaria in rice-growing areas in which cattle were scarce or absent. In Tambunan, however, this species could be accepted as having no importance in malaria transmission.

Anopheles barbumbrosus, Strickland & Choudhury.

This species is very similar to A. *barbirostris*, but breeds in rather different situations, and was not so common in Tambunan. It bred in sunshine in open grassy ravines and in clear streams after these had emerged from jungle shade.   Typical breeding places were grass-fringed streams running through narrow strips of buffalo-grazing ground between jungle-clad hills. It was easily caught in its larval stage, by dipping among the vegetation along the banks of such streams.

The adult was very rare in Tambunan. It was never captured in the human-bait trap nor by animal-bait trapping. Curiously it was one of the very few *Anopheles* captured at the Malaria Research Station, one specimen being found resting near a lighted lamp in the early evening.

Relation to Malaria.

This mosquito was so scarce that no evidence was collected in Tambunan regarding its feeding habits ; on this account it is not a vector locally.

Anopheles aitkeni, James.

This is a typical jungle mosquito. Four varieties were found in Tambunan District : the typical form, var. *bengalensis*, Puri, var. *palmatus*, Rod., and var. *borneensis*, McArthur. The last named is described in the preceding paper.

A. *aitkeni* bred in clear seepages and clear flowing streams under dense jungle shade, in association with decaying leaves and roots. From the following observations it will be seen that the breeding places varied somewhat. For instance, typical *aitkeni* was very rare and only collected in characteristic jungle seepages on three occasions, and in association with A. *a. bengalensis*. The variety *bengalensis* was by far the most common. It was found, like the others, in clear water in dense jungle shade, in association with dead leaves, sometimes in small forest pools and seepages in association with A. *leucosphyrus*, and sometimes in forest streams in association with A. *a. borneensis*. The variety *palmatus* was not taken in very close proximity to Tambunan Plain, but was found in considerable numbers at Purutan, 12 miles from Tambunan, in heavy jungle. It occurred in streams in the more remote and therefore heavy jungle surrounding Tambunan, and in clear flowing streams, and, like the others, in dense shade in association with dead leaves. The variety *borneensis* was not common and only occurred in Tambunan. It could be found constantly in certain jungle streams generally associated with A. *a. bengalensis*, always in clear water flowing over stones and rocks, with decaying vegetation and roots.

The collection of larvae was easy when search was made in the correct place, as they were not shy and did not dive, but adhered strongly to roots, decaying vegetation and stones. Dipping was carried out among roots and decaying leaves at the sides of pools or along the banks of streams, or, in seepages, as for *A. leucosphyrus* by scooping up the entire contents from a pocket of water in a seepage.

The adult of *A. aitkeni* was a small, dark, completely undecorated mosquito which assumed a marked culex-like attitude while at rest, and was therefore easily overlooked unless care was taken to distinguish it from *Culex* mosquitos. The adults of all four types of *A. aitkeni* appeared to be indistinguishable externally, although they differed in some of their minor characteristics and in their male terminalia. Adults were never taken in the human bait trap, by animal trapping, or in any other way.

Relation to Malaria.

A. aitkeni has been noted by Swellengrebel and de Graaf (1920) as occurring in highly malarious areas, and therefore not to be entirely neglected as a possible carrier of malaria. In Tambunan, the distribution of the different types of *A. aitkeni* corresponded to the distribution of the highest spleen rates, even more closely than did that of *A. maculatus* ; it was never captured, however, in association with either man or domestic animals, and it was unlikely that it had any relation to human disease. On the other hand, the distribution of *A. leucosphyrus*, the vector, corresponded very closely to that of *A. aitkeni*, and this may explain why the latter has been suspect.

A. aitkeni in all its types, therefore, was considered to have no relation to malaria in Tambunan, and the direct evidence regarding *A. leucosphyrus* was held to exonerate *A. aitkeni* from any suspicions that may have rested upon it from the epidemiological findings.

Anopheles karwari, James.

Of the *Anopheles* taken, this species formed 1 per cent. of the larval and adult captures. It bred in the same conditions, and often in association with *A. maculatus*, in open sunlit ravines, in seepages and small streams, and was generally taken when dipping for *A. maculatus*. It has been found naturally infected on rare occasions in other parts of the world, once in India, and twice in Malaya (Gater, 1935) in large series of dissections. It has, therefore, an innocent reputation, and was never seriously suspected as a vector in Tambunan, either on account of its distribution, or of its association with man. It appeared to have a preference for animals, was negative in a series of 102 dissections, and was rather rare. It had almost certainly no place as a vector.

Anopheles tessellatus, Theobald.

This mosquito was taken on rare occasions as a larva, and as an adult feeding on cattle, but never in association with man. Owing to its rarity its habits were not known, but it cannot have any importance in malaria transmission in Tambunan though it is a vector in Formosa and elsewhere.

Anopheles ludlowi, Theobald.

A. ludlowi was first described in the Philippine Islands, but the name was later for a long time wrongly applied to a brackish-water breeder responsible for much malaria in coastal areas of Malaya, Java, and elsewhere.

It is, however, a fresh-water-breeding mosquito, which shows certain differences from the brackish-water breeder more correctly named *A. sundaicus*, Rodenwaldt,

and from the very similar *A. litoralis*, King, which is found in the Philippines and Borneo.   Gater (1935) states that it is only known from the Philippines and Formosa, and probably Borneo.

Its presence in Borneo was confirmed by trapping a single female (deposited in the Institute for Medical Research, Kuala Lumpur) taken feeding on a pony at 10 p.m., at Purutan, a heavily jungled area at an altitude of about 2,000 feet, 25 miles from the sea and 12 miles from Tambunan.   No further adults and no larvae were ever found.   Because of its rarity in the locality, it could have no relation to malaria.

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ON THE DISTRIBUTION AND CORRECT NAME OF *OSCINIS PALLIPES*, THE SWARMING GNAT OF THE SUDAN.

By Curtis W. SABROSKY.

Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture.

In 1923, under the name *Oscinis pallipes*, C. G. Lamb described a tiny black fly from Khartoum, Sudan. In the accompanying notes by Cottam (1923), this fly was said to swarm in immense numbers on ceilings and upper parts of rooms and verandahs in September to December. The swarms were said to be larger than those frequently recorded in Europe for " *Chloropisca circumdata* Meigen " which is now known as *Thaumatomyia notata* (Meigen). While it is not primarily an eye gnat, as are the *Hippelates* flies in the Americas, Cottam noted that the flies " are attracted to artificial light at night, and constitute a nuisance by swarming round lamps or on dinner tables and getting into people's eyes ".

It has long been recognised that the name *Oscinis pallipes* is preoccupied, but it has never been renamed. In reviewing a number of described species, I have concluded that *pallipes* Lamb is the same form as that described by Duda from Palestine as *Oscinella sziládyi* var. *aharonii*. Typical *O. sziládyi* Duda, described from Bulgaria, has black halteres and is certainly a different species, for in my experience the haltere color is a specific and not a varietal character. The synonymy of the Near Eastern species is as follows :—

Oscinella aharonii Duda

Oscinis pallipes Lamb. In Cottam, 1923, Lancashire & Cheshire Naturalist, **15,** p. 101. (Khartoum, Sudan.) Preoccupied by *Oscinis pallipes* Loew, 1863, Berl. ent. Z., **7,** p. 37. **(Syn. nov.)**

Oscinella sziládyi var. *aharonii* Duda, 1933. In Lindner's Fliegen Palaeark. Region, Lief. 68, p. 95. (Palestine.)

Available material indicates that this species has a wider distribution than previously supposed. A series of 81 specimens (48♂♂, 33♀♀), recently received for determination through Dr. F. van Emden of the Commonwealth Institute of Entomology, was collected in West Punjab, India, 10.xi.47 (*A. W. Khan*) " swarming on roof of verandah ". Two other specimens (male, 1915 ; female, 18.viii.31) before me are from Lyallpur, Punjab. A few specimens have been seen from Iraq, and it has been sent in for determination on numerous occasions from various localities in Egypt and the Sudan, almost always with a notation that it was swarming in houses. No doubt the species will be found in many other places in the Near East under similar conditions.

The common " eye fly " of India and the Orient, *Siphunculina funicola* (Meijere), is also known to swarm in large numbers in houses. It is superficially quite similar to *aharonii*, especially in size, black body, form of the head, and polished frontal triangle, but differs in having polished thorax, distinct wing venation, and black halteres. *Oscinella sziládyi*, which is known only from Bulgaria, also has black halteres, but otherwise is more like *aharonii*. The three species are easily distinguished as follows :—

1. Second vein unusually short, the second costal sector (between tips of first and second veins) only half the length of the third sector ; mesonotum shining and polished black, not pollinose ; all coxae, femora and hind tibiae black ; halteres black..*Siphunculina funicola* (Meijere)

Second vein long, the second costal sector twice the length of the third sector ;
  mesonotum subshining, but not polished, finely pollinose ; legs pre-
  dominantly yellow **2**

2. Halteres yellow............*Oscinella aharonii* Duda (=*Oscinis pallipes* Lamb, preocc.)
   Halteres black...*Oscinella szilidyi* Duda

Inasmuch as the tiny black flies of the genus *Oscinella* are always difficult to
differentiate, it is of interest to note a peculiarity of *O. aharonii* that may be useful
in separating it from superficially similar forms. In the males, midway on the postero-
dorsal surface of the middle femur, is a small, black, wart-like elevation. Because of
its color, it is especially distinct in individuals with pale femora, but it is quite
evident even in those with browned femora. Under high magnification this elevation
appears to bear about eight short, peglike hairs with enlarged bases, and it seems
reasonable to suppose that some sensory function is involved. This structure on
the mid femur is not found in *Siphunculina*, nor do I recall ever having seen it before
in other Chloropids. Having no material of *sziládyi*, I cannot say whether it occurs
in that species.

THE BIOLOGY OF *LAEMOPHLOEUS MINUTUS* OLIV. (COL. CUCUJIDAE).

By R. G. Davies.
Imperial College of Science & Technology.

Samples taken from bulk wheat that was heating as a result of insect infestation have revealed the association of species of *Laemophloeus* with the hot regions (Howe, 1943 ; Oxley & Howe, 1944 ; Lucas & Oxley, 1946). As comparatively little was known of the biology of this genus and its rôle in the deterioration of stored food-stuffs, the present investigation was undertaken in 1940 and 1941. Circumstances prevented the study from being as complete as it might be but it is hoped that an account of the results obtained will be of value to others engaged on more extensive work on the subject.

Reid (1942 a & b) considered the three species of *Laemophloeus*—*L. minutus*, Oliv., *L. turcicus*, Grouv., and *L ferrugineus*, Steph.—that are commonly found infesting stored foodstuffs and indicated satisfactory criteria whereby the adults could be distinguished. Since then, Lucas & Oxley (1946) and Howe (1947, private communication) have found wheat infested by another species, as yet unidentified, very similar to *minutus* externally and distinguishable from it only by certain characteristics of the genitalia. In view of the close external similarity between them, it is possible that some records of *L. minutus* appearing in the literature really refer to the unidentified species. It is known, however (Howe, *loc. cit*), that the stocks of *minutus* obtained from the Department of Scientific and Industrial Research Pest Infestation Laboratory, Slough, Bucks, for the present work were correctly named, so there need be no doubt of the identity of the species under discussion here.

Distribution and Foodstuffs infested.

The genus has a wide geographical distribution and the three species mentioned above have been reported from a wide variety of stored foodstuffs as indicated by the following records :—

Laemophloeus minutus.

Wheat. Reported from most of the states of the U.S.A. by Cotton (1938), Swenk (1922) and Cooley (1922–23) ; Mexico (Strong, 1921) ; San Jacinto (Ramirez, 1921) ; Porto Rico (Wolcott, 1922) ; the Philippines (Maskew & Strong, 1920) ; the Kiangsi Province, China (Hsiu, 1936) ; Huddersfield, Gt. Britain (personal observation, 1942). Munro & Telford (1941) refer to this species as the commonest insect infesting wheat-bins and elevator storages in North Dakota.

Flour. Reported by Munro (1940) from Gt. Britain in a web produced on flour by larvae of *Ephestia kühniella*.

Maize. Finland (Linnaniemi, 1920) ; S. Paulo, Brazil (Zacher, 1932).

Rice. Louisiana (Stracener, 1934) ; Japan (Kuwayama, 1928) ; Hong Kong (Herford, 1939).

Barley. India (Ghosh, 1925).

Cotton-seed. U.S.A. (Bissell, 1936).

Copra. U.S.A. (Corbett, 1933).

Coffee-berries. Uganda (Hargreaves, 1923).

Bulbs.   Virginia, U.S.A. (Virginia State Crop Pest Commission, 1920).

Nutmegs.   In a London warehouse on a consignment from Granada (Richards & Herford, 1930).

Gum Damar.   In a London warehouse (Richard & Herford, 1930).

Laemophloeus ferrugineus.

Wheat.   Moscow (Shmal'ko, 1939) ; S.W. Ontario (Stirrett & Arnott, 1933) ; Australia (Hewitt, 1920) ; the Don Province, U.S.S.R. (Zvierezomb-Zubovsky, 1918) ; Huddersfield, Gt. Britain (personal observation, 1942).   Stored cereals (unspecified) : Formosa (Takahashi, 1937) ; Poland (Ogijewicz, 1934).

Flour.   Moscow (Shmal'ko, 1939) ; Gt. Britain (Munro, 1940).

Oil-seeds.   U.S.S.R. (Belyaev & others, 1932).

Cassava root.   Germany (Zacher, 1932).

Dried fruits.   U.S.S.R. (Arkangel'skiĭ, 1931) ;   in a London warehouse on produce from Greece (Richards & Herford, 1930).

Chillies.   In a London warehouse on produce from Mombasa (Richards & Herford, 1930).

Bean-cakes.   Japan (Takizawa, 1935).

Gum Damar.   In a London warehouse (Richards & Herford, 1930).

Laemophloeus turcicus.

Copra.   W. Africa (Passmore, 1931).

Cacao, Chillies and Nutmegs.   In a London warehouse (Richards & Herford, 1930).

There are also many records of unidentified species of *Laemophloeus* infesting foodstuffs mentioned in the above lists as well as palm-kernels (Alibert, 1938), dates (Ramachandra Rao, 1922 ; Wimshurst, 1920), dari-seed (Munro, 1940) and dried bananas (Anon, 1929).   The only other species reported from stored foods is *L. janeti,* Grouv. which Ghesquière (1922) found attacking rice, cotton-seed, flour, coconuts and cacao pods in the Belgian Congo.

Developmental Stages of *L. minutus.*

Very little has been published on the life-history and developmental stages of any of the species of *Laemophloeus.* G. V. B. Herford (unpublished thesis, University of Minnesota, 1931) has given a summary description of the life-cycle of *L. turcicus* ; Perris (1877) gives a short account of the larvae of several species ; Böving & Craighead (1931) figure and describe very briefly the head-capsule of the larva of *L. bigultatus* and there is a rather more detailed description of the external features of the larva of *L. ferrugineus* by Olliffe (1882). Zacher (1930) describes the egg of *L. minutus* very briefly.   There do not appear to be any published accounts of the prepupal and pupal stages, nor has the internal anatomy of any of the species been investigated.

The Egg.   This is a small translucent botuloid structure, the chorion devoid of external sculpture and without an obviously specialised micropylar region.   The eggs are deposited singly and, when fresh, their surface is slightly moist so that particles of the oviposition medium adhere to it.   The eggs are generally deposited in the flour or finely comminuted debris associated with the infested foodstuff.   Slight external indications of polarity are evident, the egg tapering a little more markedly at the posterior end while the ventral surface is somewhat flattened.   Eclosion is preceded by obvious embryonic movements and the appearance of an iridescent sheen on the

surface of the shell as air enters the egg when the amniotic fluid is absorbed by the embryo (*cf.* Sikes & Wigglesworth, 1931). The chorion ruptures a little behind the anterior end and the first-stage larva emerges through the irregular rent thus formed, taking about fifteen minutes to effect complete withdrawal from the shell.
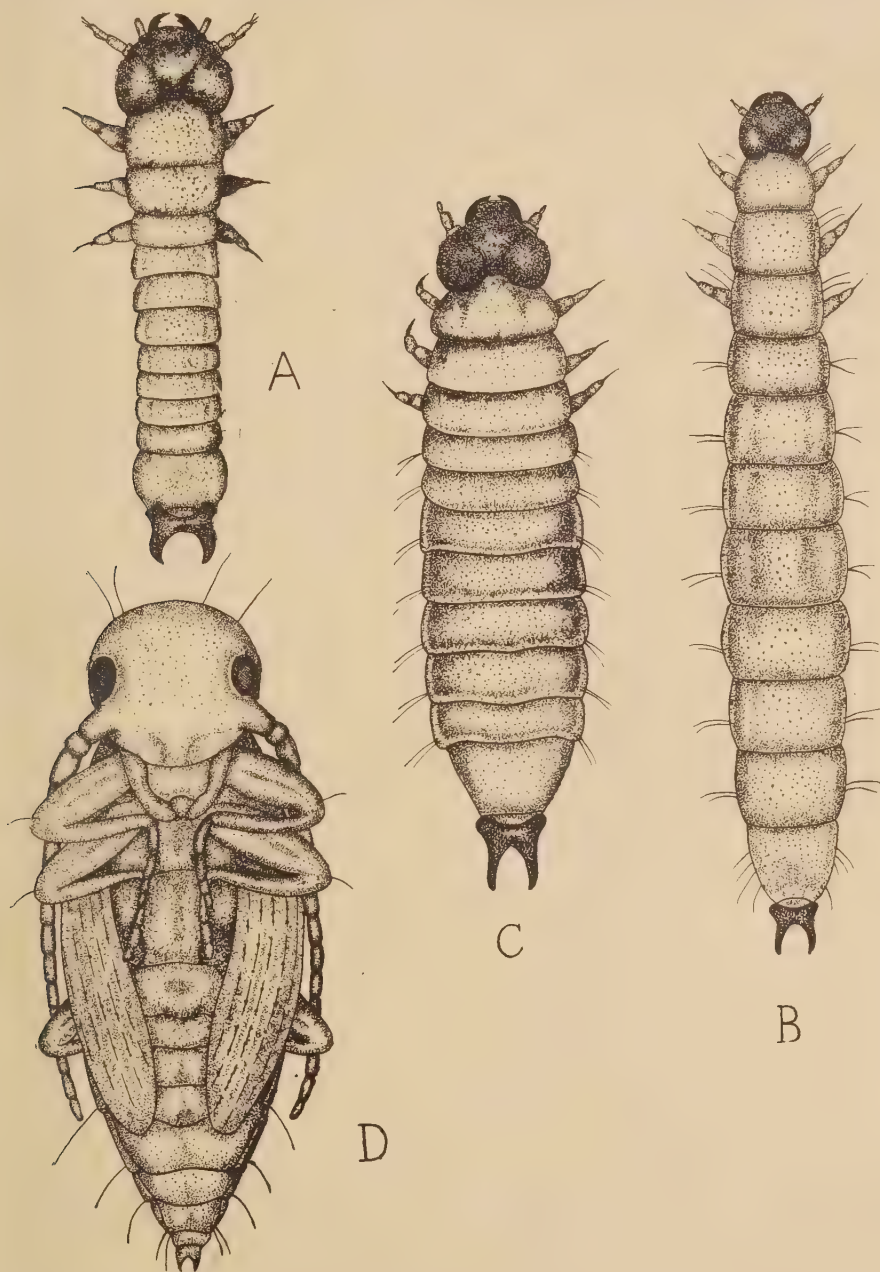
Fig. 1.—*Laemophloeus minutus.* A, first-stage larva (×90). B, active fourth-stage larva (×30). C, fourth-stage larva, prepupal phase (×45). D, pupa (×50).

E

First Larval Instar. The first-stage larva is quite active on emergence from the egg, with all its setae erect and a completely transparent exoskeleton, although sclerotisation of the head-capsule and anal hooks occurs within twenty-four hours. In form this instar is rather elongate with a depressed body, the dorsal surface slightly convex and the ventral surface flattened. The head-capsule is flattened, transverse and sparsely covered with fine setae. The postero-dorsal edge of the head-capsule is strongly emarginate and anteriorly there is a small semi-circular labrum beneath which mandibles and maxillae are evident. The antennae are three-jointed—a short, broad, basal segment and two sub-equal longer ones, the terminal segment being narrowest and bearing a tuft of setae distally. There is a single group of lateral ocelli on each side of the head.

The three thoracic segments are well-defined, the most anterior being broader and longer than the others, while the metathorax is smallest. Each is rounded at its basal and apical angles and there is a sparse distribution of setae. The three-segmented legs terminate in a sharply pointed claw which curves ventrally. The abdominal segments of the first instar are of approximately equal breadth and the first seven are each about one-half as long as the prothoracic segment. The eighth segment is considerably longer than the others and its posterior angles are markedly obtuse. The ninth segment is much shorter than the remainder and bears a pair of anal hooks (urogomphi) which curve inwards and slightly dorsalwards. The anus is placed ventrally on the ninth segment and is preceded on the eighth by a horse-shoe shaped sclerotisation. With the exception of the head capsule and the anal hooks the integument is soft and unpigmented with the white fat-body visible through it. There is a single pair of metathoracic spiracles and a pair on each of the first eight abdominal segments.

Later Larval Instars. There are four larval instars in all, a part of the fourth stadium being spent as a quiescent prepupa. The number of instars is remarkably constant for a beetle, only one supernumerary moult being noted in the many hundreds of individuals reared during the course of this work. The second, third and fourth larval instars are distinguished from the first instar not only by their size, but also by changes in relative proportions. The thorax and abdomen are relatively less broad in the active fourth-stage larva while the body length increases more than that of the head capsule and urogomphi. The appearance of the first and fourth instar is shown in fig. 1, while the structure of the mouth-parts of the fourth instar is illustrated in fig. 2.

A comparison of a small number of fourth-stage larvae of *L. minutus*, *L. ferrugineus* and *L. turcicus* showed the former species to be distinguishable from the other two by its more pronounced emargination of the postero-dorsal edge of the head-capsule, by the fact that its anal hooks are only about 1·5 times their combined breadth, whereas in *ferrugineus* and *turcicus* they are almost twice as long and, finally, by the anterior part of the horse-shoe shaped sclerotisation on the ventral surface of the eighth abdominal segment in *minutus* being so slender as almost to result in the sclerotisation appearing as a pair of curved bars. No satisfactory criteria were found for separating *turcicus* and *ferrugineus* larvae.

After a period of active feeding the fourth-stage larva begins to prepare the cocoon in which pupation later occurs. In assuming the prepupal phase (fig. 1) the larva becomes relatively more broad in form and somewhat sluggish in behaviour while the antero-ventral part of the prothorax becomes enlarged through the development of a pair of mammiliform silk glands. These rounded conical elevations bear at their apices a group of small apertures through each of which issues a single strand of silk. The anterior position of the silk glands appears to be unique among coleopterous larvae since all other known forms (Wigglesworth, 1939) produce silk in

modified Malpighian tubules and extrude it through the anus.   The cocoon is ovoidal, often spun against a smooth surface within the larval food and with fragments of grain-husks, etc., incorporated in its outer surface.

Pupa.   After a short time the quiescent prepupa moults within the cocoon to give the pupal stage.   This is a semi-transparent exarate white form illustrated in fig. 1.   The cuticle is entirely transparent at first with the ventrally flexed head virtually continuous with the prothorax but in older pupae a dark brown pigment is deposited in the eyes and the sclerotised antennae and legs of the developing imago become evident beneath the pupal integument.   The head-capsule, mandibles (especially the incisor edge), and the prothorax later show sclerotisation and, to a lesser extent, the abdominal sternites.   The pupae of *L. minutus* and *L. turcicus* may easily be sexed since in females the antennae extend only to the metathorax, whereas in males they reach the middle of the abdomen.   Mature pupae of *L. ferrugineus* differ from those of the other two species found in stored products in that the basal edge of the prothoracic sclerotisation beneath the pupal integument is only about half as long as the apical edge—the prothorax of the adult *L. ferrugineus* being almost cordiform while that of *minutus* and *turcicus* is quadrate.
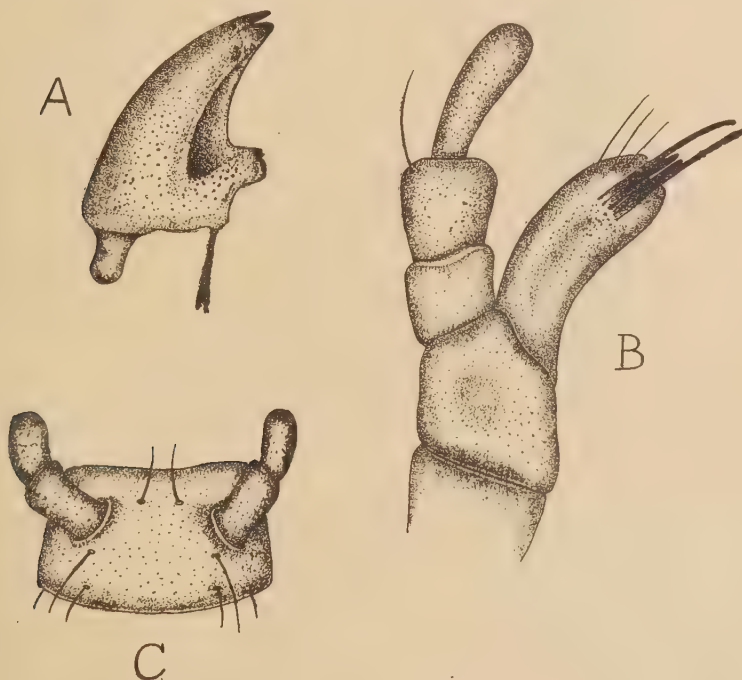
Fig. 2.—Mouth-parts of fourth-stage larva of *Laemophloeus minutus.*   A, mandible ($\times 250$).   B, right maxilla, ventral aspect ($\times 400$).   C, Labium ($\times 400$).

Physical Ecology of *L. minutus.*

A knowledge of three factors is of prime importance in understanding the build-up of large insect populations : firstly, the rate and duration of oviposition ; secondly, the rate of development from egg to adult and, thirdly, the degree of mortality in the developing population.   The greater part of the investigation described here

was devoted to determining these quantities under various controlled conditions of temperature and humidity, on the assumption that temperature and humidity changes in, say, the intergranular atmosphere of bulk wheat are the most important factors affecting the development of infestations.

Oviposition.

(a) *Onset of oviposition in mated females.*

A supply of virgin females, freshly emerged, was obtained from stock cultures and each paired with a young male in a 2 in. $\times \frac{1}{2}$ in. specimen tube on an oviposition medium of whole-meal flour ground sufficiently fine to pass through a sieve of 80-mesh bolting silk.   Tubes were kept at 25°C. and 75 per cent. Relative Humidity and their contents sieved at 4-day intervals to determine when oviposition started. The results are illustrated in fig. 3 which shows that the majority (63·8 per cent.) of females started laying within four days of emergence and that almost all (91·3 per cent.) were laying within twelve days.
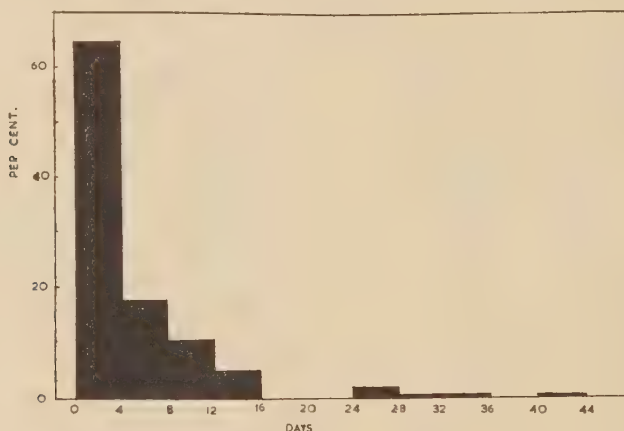
Fig. 3.—Frequency diagram showing percentage of females starting to lay eggs within various periods after emergence.

(b) *Course of oviposition in presence of males.*

By recording the numbers of eggs laid during each 4-day period for the pairs described above and continuing until the female died or oviposition ceased, data on the length of the oviposition period at 25°C. and 75 per cent. R.H. were obtained for 24 individual females.   Table I gives full details of the duration of egg-laying life, while Table II is a grouped frequency table of duration.   This latter table is peculiar in that it suggests females lay either for a short period (75 days or less) or for a long one (more than 125 days) intermediate values being very few.   It is probable, however, that a larger number of females would need to be used to decide whether this effect was a genuine one or simply due to sampling error.

Oviposition records of four typical females are shown diagrammatically in fig. 4. There is considerable variation in the rate of egg production at various times during the oviposition cycle, but no evidence of a marked peak and only a slight indication of a decline in fecundity towards the end of the cycle.   In every case, except the two mentioned in connection with Table I, the records were terminated by the death of the female concerned.   This type of oviposition cycle in which a few eggs are laid daily over a long period is characteristic of most coleopterous pests of stored

products—e.g. *Tribolium confusum* (Dick, 1937 ; Good, 1933) ; *Gnathocerus cornutus* (Morison, 1925) ; *Necrobia rufipes* (Howard, 1924) ; *Calandra granaria* (Back & Cotton, 1926) ; *Tenebrio molitor* (Dick, 1937) ; *Dermestes vulpinus* (Dick, 1937) ; *Calandra oryzae* (Birch, 1945) ; *Rhizopertha dominica* (Birch, 1945) and *Tribolium destructor* (Reynolds, 1944). It contrasts with the other types of oviposition cycle in the Coleoptera discussed by Dick (1937).

TABLE I.

Duration and intensity of oviposition by *L. minutus* females in presence of males.

Index No.	Oviposition period (days)	Total eggs laid	Daily average
M1	45	110	2·4
M2	37	74	2·0
M3	49	110	2·3
M4	101	179	1·8
M5	125	301	2·5
M6	23	41	1·8
M7	69	167	2·4
M8	128	153	1·2
M9	149	215	1·5
M10	239*	509	2·1
M11	204	341	1·7
M22	194	444	2·3
M25	164	274	1·7
M30	212	225	1·1
M31	20	48	2·4
M28	160	424	2·6
M36	108	282	2·6
M38	182	463	2·5
M39	182	289	1·5
M41	182	131	0·7
M45	167	216	1·3
M47	167	374	2·2
M48	24	92	3·8
M54	206*	345	1·7

Average total eggs laid = 242 ± 28·1
Daily average laid = 1·85 ± 0·14

*Female still laying when experiment had to be discontinued. Values of 239 and 206 respectively used in computing averages.

TABLE II.

Frequency distribution of length of oviposition period.

Duration (days)	No. of females
0–24	3
25–49	3
50–74	1
75–99	0
100–124	2
125–149	3
150–174	4
175–199	4
>200	4

(c) *Effect of males present.*

The relation of fecundity to mating varies considerably among the Coleoptera. In the Dermestid, *Trogoderma versicolor*, Norris (1936) found that, providing copulation had occurred once, isolated females laid as many eggs as those with which a

male was constantly present, while Reynolds (1944), working with *Tribolium destructor*, noted a significant increase in the rate of egg-production after males had been removed. On the other hand, in the Cerambycids, *Tetropium gabrieli* and *T. fuscum* (Schimitschek, 1929), in *Tribolium confusum* (Park, 1933) and in *Bruchus quadrimaculatus* (Brauer, 1944) repeated copulation occurs and is apparently necessary to maintain a high rate of egg-production.
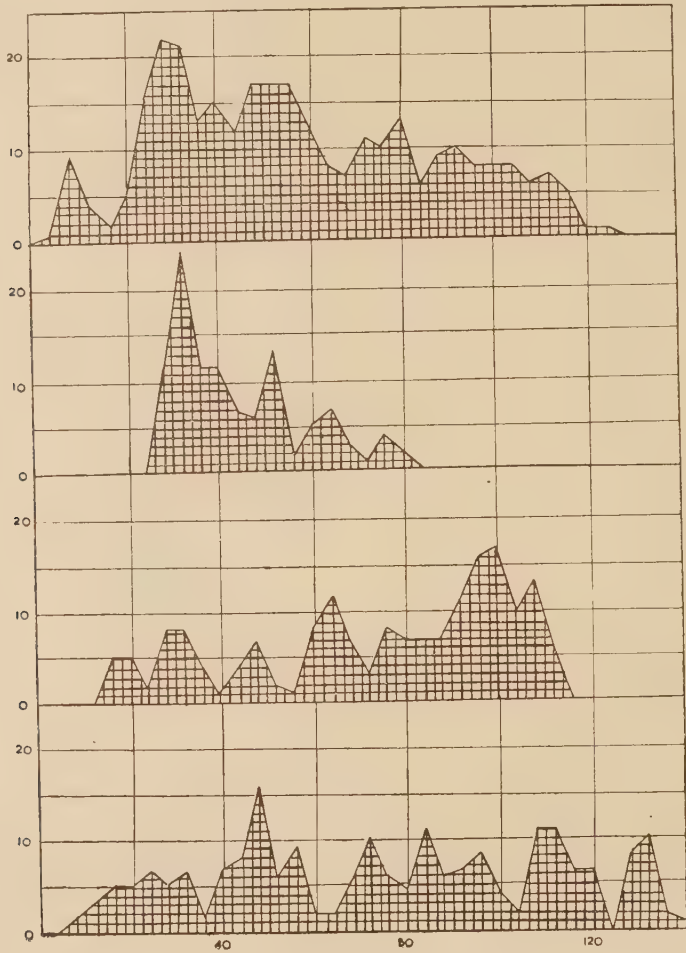
Fig. 4.—Oviposition records of four females of *Laemophloeus minutus*. Ordinates show number of eggs laid within each four-day period and abscissae indicate age of female in days.

In order to examine the conditions obtaining with *Laemophloeus minutus*, virgin females were confined separately on an oviposition medium, each with a male, for 30 days and oviposition records taken; the males were then removed and records continued to be taken. A control series in which males were left with the females throughout was also recorded. The results are not very easy to interpret owing to the considerable variation between females and between different parts of the

oviposition cycle of a given female, but the summarised data of Table III indicate
that the presence of the male results in a considerably higher rate of egg-production.

(d) *Effect of various temperatures and relative humidities.*

In view of the variability in the rate of oviposition it was felt that no great
reliance could be placed on a simple comparison of the results of oviposition tests in
which small groups of mated females were each kept at a different temperature and
relative humidity from the beginning of the experiment. Such a technique might
have yielded satisfactory data if very large numbers of females were used at each set
of conditions, but if—for reasons of time—groups of, say, ten females were to be

TABLE III.

Oviposition in presence and absence of males.

Experimental			Controls Male present throughout		
	Eggs per day			Eggs per day	
Index	1st–30th day Male present	30th day onwards Male absent	Index	1st–30th day	30th day onwards
MR13	1·0	0·1	M5	2·9	0·9
MR14	2·9	0·7	M11	0·2	2·1
MR15	1·5	0·1	M41	1·5	0·4
MR16	0·7	0·3	M36	2·6	2·7
MR18	0·5	0·2	M7	2·5	2·4
MR21	2·2	2·0	M47	2·8	2·8
Means	1·4	0·6	Means	2·1	1·9

used, then sampling errors might well mask any effects due to environment. Accord-
ingly it was decided to record oviposition in a " standard " environment of 25°C.
and 75 per cent. R.H. for a period of 24–28 days and then transfer sets of females
from the standard conditions to various combinations of temperature and humidity.
Oviposition records were then continued in the new environment and its effect
assessed by comparison with the standard. A somewhat similar technique was
used by Dick (1937) though it does not, of course, eliminate the variability known
to exist between different parts of the same insect's oviposition cycle. The results
obtained are summarised in Table IV which also gives the probability that the
observed difference between standard and experimental environments can be
ascribed to chance (evaluated by means of the well-known t-test of significance).

TABLE IV.

Effect of temperature and relative humidity on oviposition rate.

Experimental environment	Eggs per day in experimental environment	Eggs per day in standard environment	P.
25°C., 55% R.H.	1·6	1·9	0·05–0·02
25°C., 65% R.H.	1·9	2·0	0·3–0·2
25°C., 75% R.H.	2·0	2·0	0·8–0·7
25°C., 90% R.H.	2·0	2·1	0·7–0·6
17°C., 75% R.H.	0·5	2·9	0·05–0·02
21°C., 75% R.H.	1·2	1·0	0·05–0·02
30°C., 75% R.H.	3·8	1·0	$<0·01$

It is probable that this method of assessing the effect of temperature and humidity on the rate of oviposition is not entirely satisfactory, the apparently significant increase in oviposition-rate on reducing the temperature from 25°C. to 21°C. possibly reflecting variation between different parts of the insects' laying cycles, but the data seem sufficiently convincing to suggest that humidity (over the range 55 to 90 per cent. R.H.) has little effect on the rate of egg-laying, whereas it is markedly stimulated by high temperatures (30°C.) and almost inhibited at 17°C.

Effect of Temperature and Humidity on Rate of Development.

Two techniques were employed in order to examine the relation between developmental rate and the temperature and relative humidity of the environment. In the first case, " mass-cultures " were started from 20 eggs (aged up to 24 hrs.) in each of 5 specimen tubes with an adequate supply of whole-meal flour, the tubes being incubated at the appropriate temperature and humidity. Dates of emergence of the imagines were noted and so the total duration of the life-cycle and the percentage mortality could be determined. To obtain data on the duration of the separate stages of the life-cycle, however, a method of " individual cultures " was required, whereby the developing insects were isolated and easily accessible to frequent periodic examination. This was achieved by rearing the insect from the egg in cylindrical glass cells containing a thin layer of whole-meal flour. Each cell (measuring 10 mm. in diameter and 12 mm. high) contained a single egg and 25 such cells were cemented to a glass plate for convenience in handling and storage in tiers in desiccators containing the appropriate humidifying solutions. The results obtained by these techniques are summarised in Table V. This table shows the mean duration, in days, of each stage. The results for the mass-cultures are shown in the extreme right-hand column of the table for comparative purposes.

Effect of temperature and humidity changes on mortality.

Mortality records for each individual stage were kept for the individual cultures and these percentages, together with the over-all mortality data from the mass-cultures (i.e., adults emerging per 100 eggs used) are shown in Table VI.

General discussion of rate and mortality data.

It is not proposed here to give in full the statistical treatment of the data presented in Tables V and VI. The small size of some of the samples occasionally prevents the demonstration of a significant effect of temperature and humidity changes on the mortality or rate of development, but after consideration of the results of applying the appropriate statistical tests (involving the computation of " t " or Chi-squared) the following conclusions emerge from the data relating to individual cultures :—

(i) Temperature changes profoundly affect the rate of development of all stages of the life-cycle, the optimum temperature in every stage lying near, and probably just below, 35°C. Samples are of adequate size to demonstrate this unequivocally. The temperature threshold of development appears to lie a little below 17°C. and above 15°C., at which latter temperature no development occurs.

(ii) Temperature has a less marked effect on mortality, though in the case of the egg the death rate at 35°C. is significantly higher than that at all lower temperatures involved (P < 0·01). The summation of small (and sometimes non-significant) differences in the mortalities for the separate stages leads, on considering the over-all death rates in individual cultures—i.e., adults emerging per 100 eggs used—to a significantly lower mortality at 25 and 30°C. than at other temperatures (P < 0·02). The relatively slight effect of temperature changes on the mortality of some developmental stages means that larger samples than those here employed will be necessary if the magnitude of the effects is to be estimated accurately.

TABLE V.

Effect of temperature on rate of development of *L. minutus.*

Temperature	Relative Humidity	Egg	1st Instar	2nd Instar	3rd Instar	4th Instar (excl. prepupa)	Prepupa	Pupa	Egg-Adult (Individual)	Egg-Adult (Mass-culture)
						Duration of stage in days				
17°C. … … …	55%	18·3	—	—	—	—	—	—	—	—
	65%	20·2	21·0	—	—	—	—	—	—	137·0
	75%	19·6	23·5	16·5	—	—	—	—	—	137·4
	90%	15·4	23·3	16·6	13·7	18·2	15·8	16·7	116·1	—
21°C. … … …	55%	10·2	21·5	19·5	20·1	23·2	14·5	15·8	112·5	—
	65%	11·3	21·8	20·5	15·5	15·0	12·0	16·0	103·0	93·8
	75%	10·7	15·3	12·9	11·8	12·8	12·0	13·5	89·5	79·4
	90%	12·0	13·1	8·2	9·1	10·3	8·3	11·9	67·7	—
25°C. … … …	55%	6·8	14·8	11·1	9·7	12·2	7·9	7·9	64·7	57·2
	65%	6·3	10·9	9·3	9·1	9·8	5·8	7·6	56·4	44·2
	75%	6·4	8·8	6·6	8·3	8·9	5·5	7·8	47·4	44·4
	90%	6·2	5·8	4·3	4·4	4·9	12·0		34·8	—
30°C. … … …	55%	4·5	15·3	8·8	7·2	10·5	5·5	7·5	48·5	47·0
	65%	4·6	6·4	4·1	4·4	4·6	3·3	5·0	29·8	33·0
	75%	4·0	5·8	3·9	3·9	5·0	3·2	4·9	27·9	29·0
	90%	4·5	4·0	3·4	3·6	3·1	3·2	5·0	24·8	—
35°C. … … …	65%	6·2	7·2	3·8	3·8	4·2	3·8	4·5	30·5	—
	75%	3·9	5·6	3·2	2·8	7·2	4·5	5·5	28·5	—

TABLE VI.

Effect of temperature and relative humidity on mortality.

Percentage Mortality at various stages

Temperature	Relative Humidity	Egg	1st Instar	2nd Instar	3rd Instar	4th Instar (excl. prepupa)	Prepupa	Pupa	Over-all	Over-all (mass-cultures)
17°C.	55%	14·8	100·0	—	—	—	—	—	100·0	100·0
	65%	9·6	80·8	100·0	—	—	—	—	100·0	99·0
	75%	17·4	98·5	—	—	—	—	—	100·0	95·0
	90%	4·3	24·4	26·7	38·1	7·5	27·3	0·0	86·8	—
21°C.	55%	11·0	76·6	37·5	12·5	20·0	0·0	25·0	96·2	100·0
	65%	5·7	97·2	0·0	0·0	0·0	0·0	0·0	98·3	36·0
	75%	5·6	91·0	54·5	40·0	0·0	0·0	0·0	99·3	14·0
	90%	3·9	21·1	6·7	10·7	0·0	16·0	5·9	54·3	—
25°C.	55%	9·3	86·9	37·7	22·2	14·3	16·7	0·0	96·0	39·0
	65%	3·3	77·5	9·5	10·5	0·0	6·3	6·7	90·5	40·0
	75%	7·5	72·7	10·3	3·6	9·5	11·1	6·6	89·3	29·0
	90%	0·0	8·8	0·0	3·6	0·0		12·0	26·7	—
30°C.	55%	13·8	86·5	20·0	25·0	33·3	0·0	50·0	97·9	38·0
	65%	8·0	13·3	13·0	5·3	0·0	7·1	7·7	48·1	24·0
	75%	3·9	15·8	0·0	0·0	14·3	8·3	0·0	45·0	7·0
	90%	2·0	7·5	11·1	0·0	0·0	14·3	0·0	53·8	—
35°C.	65%	42·5	84·0	0·0	0·0	0·0	0·0	0·0	93·3	—
	75%	42·9	36·0	0·0	0·0	0·0	50·0	0·0	97·0	—

(iii) The effect of humidity on the rate of development is also apparent over the range employed here though in the case of the egg-stage (where samples were large) there is almost certainly no effect. In other stages there is evidence that decreasing the relative humidity results in a lower rate of development, but not all such observed differences proved significant at a probability level of $P=0\cdot05$.

(iv) Humidity changes have a more pronounced effect on mortality, particularly in the case of the first-stage larva where a fall in R.H. from 90 per cent. to 75 per cent. leads to a greatly increased death rate. The effect of variations in relative humidity is least evident at 17°C. where mortality is generally higher than at the remaining temperatures.

A comparison of the data obtained by the method of individual culture with those from the mass-cultures shows fairly satisfactory agreement in respect of the rate of development, though the life-cycle tends on the whole to be rather shorter in the mass-cultures. It is, however, obvious that mortality in the mass-cultures is far lower than that in the individual cultures. It was not possible to initiate experiments to determine the cause of this difference, but possible explanations suggest themselves. In the first place, it is true that larvae in the mass-cultures had a large excess of food, whereas the mass of food per isolated larva was limited. However, there was no evidence to suggest that the isolated larvae were starving, as excess food was always present in the glass cells. Furthermore, in cases of food deficiency the rate of development would probably be reduced and though there was some inconclusive evidence of this at 25°C./75 per cent. R.H. and 21°C./75 per cent. R.H., the rates were similar at 30°C. but the mortalities still widely different.

A more plausible explanation is that the larvae (especially the first-instar, which is particularly susceptible to dry conditions) were able, in the mass-culture, to create—by excretion of metabolic water—a microclimate of higher relative humidity within localised zones of the flour in which they were living. The attainment of equilibrium between the water-vapour content of the atmosphere and the moisture-content of flour exposed to it is known to be a long process, indicating that water-vapour does not readily diffuse through a layer of flour of any thickness, whereas in the individual culture cells the layer of food was often thin enough for the body of the larva to be in direct contact with the atmosphere of the humidity chamber. Since the above hypothesis has only rather slender circumstantial evidence in its favour it is not proposed to discuss further its implications for the general subject of the ecology of insects inhabiting foodstuffs like flour ; the whole question of microclimates in such environments is obviously one requiring detailed experimental analysis. It will be noted that in spite of the absolute differences in mortality in the two types of culture, the variations in mortality with changes in temperature and humidity are roughly parallel.

Heat Production by *Laemophloeus.*

Extensive field experience, summarised by Oxley & Howe (1944), Howe (1943) and Lucas & Oxley (1946), has indicated that *Laemophloeus* is associated particularly with the hot regions of infested bulk wheat. The experimental results presented above show that *L. minutus* is well adapted to temperatures in the vicinity of 30–35°C. and it therefore becomes a matter of considerable practical importance to know whether *Laemophloeus* is itself capable of raising the temperature locally in bulk wheat or whether it simply migrates to those parts of the bulk which are heating as a result of the concentration there of other insect pests (*e.g.*, *Calandra*) or from some quite different cause. The fact that *Laemophloeus* can sometimes survive the winter in bulk grain in which *Calandra* succumbs (*e.g.*, in the example discussed by Howe, 1943) means that in the absence of external sources of infestation in the

following spring, an infestation which was originally mixed may become a pure *Laemophloeus* infestation—obviously a more serious matter if *Laemophloeus* could initiate grain-heating. Preliminary details of heat-production by *Laemophloeus* were therefore obtained by setting up cultures of different initial population densities in uninsulated cylindrical glass jars on crushed wheat grains (a suitable medium for rapid reproduction) and recording daily the increased temperature at the centre of the jars by accurately calibrated Beckmann thermometers. The cultures were stored at 25°C. and 75 per cent. R.H. throughout.

The results are shown graphically in fig. 5. It is probable that the decline in heat production after about 32 days is associated with the accumulation of carbon dioxide, with competition in the limited volume of grain used and with the declining fertility of the females, which were taken from a stock culture and were not of uniform age. It is immediately evident, however, that a considerable temperature rise occurs and an examination of the cultures showed that the maximum temperature reached was accompanied by the appearance in the cultures of fourth-instar larvae. Certainly, heating is due to larval activity rather than to that of the adults, for the population of adults remained approximately the same at the end of 30 days as it was initially, when there was virtually no heating.
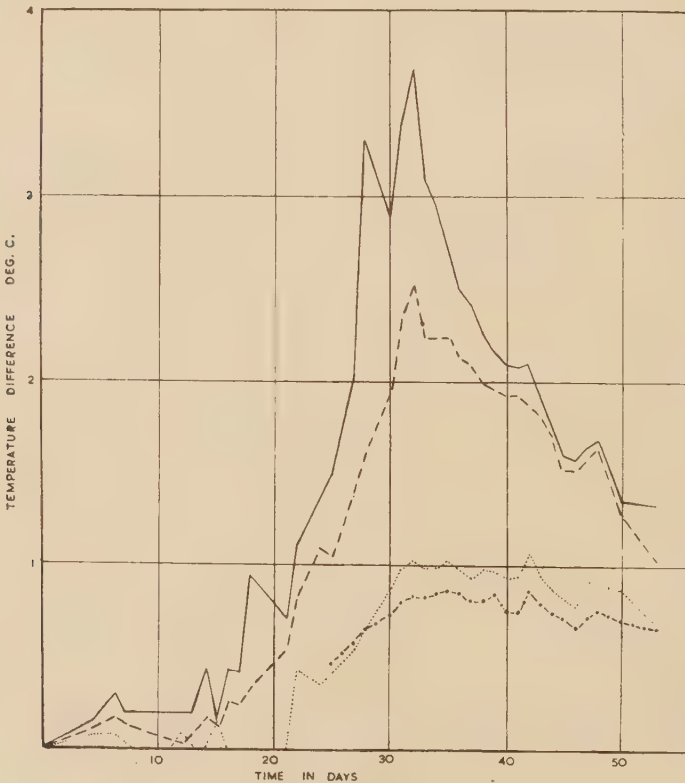
Fig. 5.—Graph showing temperature difference between environment and centre of cultures of *Laemophloeus minutus* at four different initial population densities. ————500 females per 350 cc. grain; — — —350 females; ·············150 females; —·—·—70 females.

The quantitative interpretation of data on temperature rises during infestation has recently been discussed by Oxley & Howe (1944) who draw attention to the equation of Awbery (1927) for the temperature difference between the centre and the surface of a body generating heat internally and to the relation which exists between heat-production and carbon dioxide output under controlled conditions (the so-called " CO_2 figure ", defined as the percentage by volume of CO_2 produced in 24 hours at 25°C. in the intergranular air of a sample of infested grain completely filling a closed container). An approximation to the rate of heat production at the temperature maxima of fig. 5 can be obtained by applying Awbery's equation for a sphere (the case of the cylinder, such as was used in these experiments and in which radiational heat-loss would be smaller, was unfortunately not considered by Awbery). The corresponding CO_2 figure can be calculated from an equivalence given by Oxley & Howe (1944) and the resulting data on the heat-production of *L. minutus* are given in Table VII. These figures are of the same order of magnitude as those mentioned by Oxley & Howe for *Calandra granaria*, which is generally credited with the ability to heat grain and may therefore be taken as a strong indication of the importance of *Laemophloeus* in initiating grain-heating.

TABLE VII.

Heat-production by *Laemophloeus minutus*.

Initial density (females)	Maximum rate of heat-production (cals./sec./cc. $\times 10^4$)	CO_2 figure	Days to reach maximum
500	4·4	2208	32
350	3·0	1512	32
150	1·3	642	42
70	1·0	522	42
0	0·0	0	—

The dangers of direct extrapolation of the results of these laboratory experiments to the field scale are fully realised, but it is of interest to note the implications which they have for the study of natural infestations. In the first place, it will be seen that the maximum rate of heat-production attained is approximately proportional to the initial density of females (fig. 6). Secondly, it is known (Oxley & Howe, 1944) that heating of bulk grain generally originates in relatively small zones with a local concentration of insects. By combining the relation implicit in fig. 6 with Awbery's equation it is possible to calculate the difference in temperature between the centre and periphery of a spherical zone of any given radius and for any given initial density of females. This has been done for a sphere of one metre radius and the results shown in the nomogram of fig. 7. Since there is evidence that the optimum temperature for *Laemophloeus minutus* is not above 35°C. it is clear that in grain at 25°C. (to which temperature all the rates of heat production determined refer) the insect can raise the temperature to above its optimum in a localised spherical zone of 1 m. radius when the initial density of females is about 0·7 per 100 cc. of grain. This corresponds to a total of about 29,000 females in a sphere of 1 m. radius and is considerably less than the densities which have been recorded from badly infested wheat bulks (Howe, 1943). It is believed that more extensive work on the lines indicated above would be of considerable value in predicting the likelihood of heating in stored wheat from a consideration of initial population densities.

Mode of Attack on Wheat Grains.

The heating experiments recorded in the previous section were all carried out with crushed grain on which it was known that the insects would breed readily. From a practical point of view it is of the utmost importance to know whether

R. G. DAVIES.
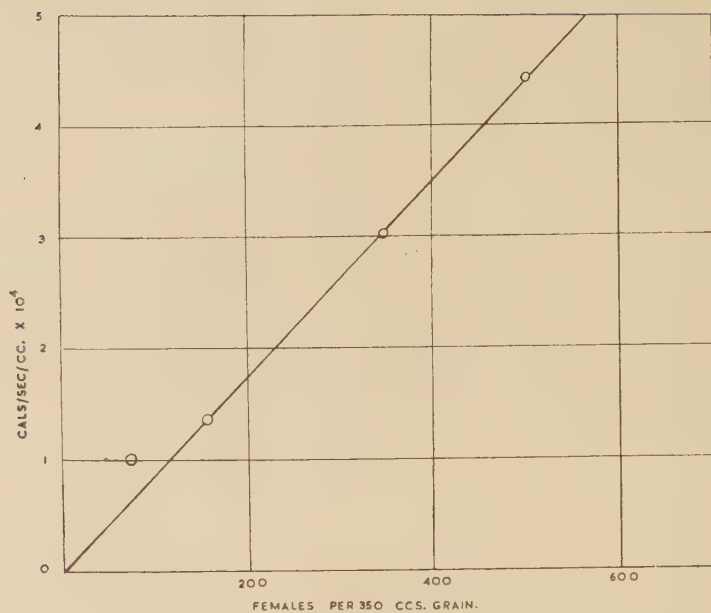
Fig. 6.—Relation between maximum rate of heat production in experimental cultures and initial population density.
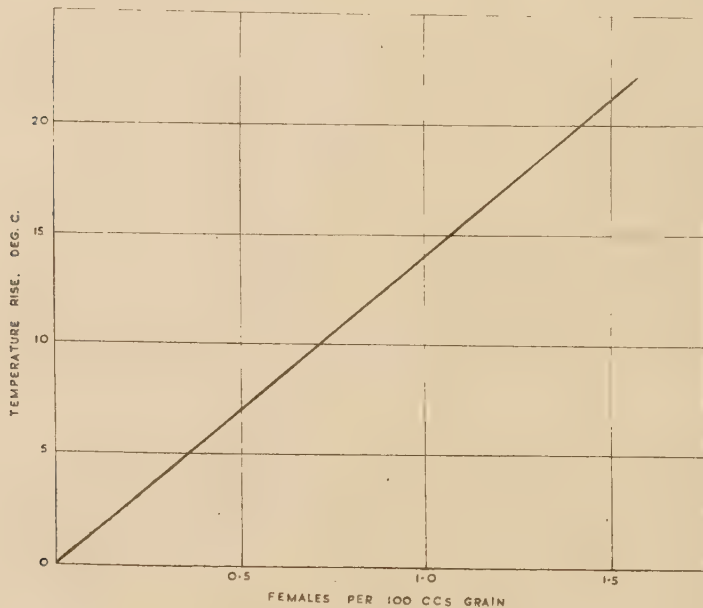
Fig. 7.—Relation between initial population density of *Laemophloeus* and equilibrium temperature difference between the centre and surface of a spherical bulk of grain of 1 m. radius.

breeding will easily occur in the apparently undamaged type of wheat which is normally put into storage—*i.e.*, to know whether *Laemophloeus* is a primary pest like *Calandra granaria* or *Rhizopertha dominica,* or whether it is only capable of breeding in, and therefore raising the temperature of, grain previously damaged by insect attack or from other causes. This topic was investigated by preparing three cultures. The first contained as food a quantity of absolutely undamaged Manitoba No. 1 wheat. Each grain used for this culture was examined under a low-power microscope and any with the slightest blemish were rejected. The second jar contained an equal volume of grain which, apparently in sound condition on a naked-eye examination, was actually made up of berries in which there was some small defect in the surface of the fused pericarp and testa. Such damage had all resulted from normal handling processes and was not the result of previous insect attack. The third jar contained an equal volume of whole-meal flour as a control. Five hundred adults (unsexed) were added to each jar and all incubated for 14 days at 25°C. and 75 per cent. R.H. At the end of the period the contents of each jar were examined with the following results :—

1. *Undamaged Grains.*

   Percentage adults dead : 91·0 ; first instars/100 adults : 0·71 ; later instars : Nil.

2. *Grains with slight blemish.*

   Percentage adults dead : 26·0 ; first instars/100 adults : 30·0 ; later instars/ 100 adults : 4·5.

3. *Flour Control.*

   Percentage adults dead : 4·8 ; third and fourth instars/100 adults : 25·1 ; earlier instars not counted, but very numerous.

These results show very strikingly that perfectly whole grains cannot provide food for the adults nor do they permit reproduction. It is possible that egg-laying is also inhibited on a medium of undamaged grain since only one malformed egg was found in the culture concerned, but it is more likely that the eggs laid were eaten by the adults. When the grains which originally had a slight blemish were examined it was found that in almost every case the original damage was accentuated by insect attack. In a very small number of cases the grain had been hollowed out ; in most cases the embryo had been consumed—the original damage was usually in the wrinkled grain-covering near the embryo—but occasionally the endosperm alone was eaten. Eggs were found on the outside of the grains or in the frass which accumulated at the bottom of the jar.

Strictly speaking, therefore, the damage done by *Laemophloeus* should be described as secondary since absolutely intact grains are immune to attack. The average sample of apparently sound commercial wheat, however, is bound to contain an appreciable proportion (say, about 30 per cent.) of berries in which some slight damage to the covering has resulted from normal handling processes. Such trivial blemishes, it has been shown, can be enlarged by *Laemophloeus* and it is therefore suggested that grain free from the well-known primary pests like *Calandra* and *Rhizopertha* may nevertheless permit an infestation of *Laemophloeus* to develop. Whether such an infestation would be sufficiently extensive or develop sufficiently rapidly to result in large-scale deterioration of the wheat is a matter which can only be settled by further investigations.

Summary.

The three species of *Laemophloeus* commonly associated with stored products have been shown, by a survey of recorded information, to be cosmopolitan and to occur on a large variety of foodstuffs.

The egg, four larval instars and pupa of *L. minutus* have been described briefly and the life-history outlined.

Mated females of *Laemophloeus minutus* lay eggs continuously throughout their long life at the rate of about 0·5 eggs per day at 17°C. rising to 4·0 eggs per day at 30°C. Humidity changes over the range 55–90 per cent. R.H. have little effect on the rate of oviposition. Oviposition is stimulated by the constant presence of the male.

A detailed study was made of the effects of temperature (17–35°C.) and relative humidity (55–90 per cent.) changes on the rate of development and mortality of each stage in the life-cycle. The mortality of egg and pupa is hardly affected by humidity changes whereas the first instar—and, to a lesser extent, the other larval stages—succumb readily to dry conditions and survive easily at 90 per cent. R.H. Temperature has little effect on mortality except for a marked reduction of the viability of eggs at 35°C. Dry conditions tend to retard development of the larval stages somewhat, but temperature is the main factor determining the length of the life-cycle, the optimum being between 30° and 35°C.

Experiments on small cultures suggest that serious heating of bulk grain might result from a pure infestation of *Laemophloeus*.

Completely undamaged grains are virtually immune from attack, but normal samples of wheat contain an appreciable proportion of grains with very small blemishes which are accentuated by *Laemophloeus* and thus rendered suitable for the insects' development.

Acknowledgements.

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CONTROL OF *EMPOASCA LYBICA*, DE BERG., ON COTTON IN THE ANGLO-EGYPTIAN SUDAN.

By J. W. Cowland, B.A.

Senior Entomologist, Research Division, Department of Agriculture and Forests, Sudan,

and

. C. J. Edwards, B.Sc.,

Harston, Cambridge.

(Plate II.)

During the past decade the Cotton Jassid, *Empoasca lybica,* de Berg., has become a pest of increasing importance on cotton grown in the northern and western blocks of the Gezira Scheme and the Northern Alternative Livelihood Schemes of the White Nile. The Gezira Scheme (fig. 1) is an irrigated area situated along the west bank of the Blue Nile and watered by gravity from the Sennar Dam. Approximately 170,000
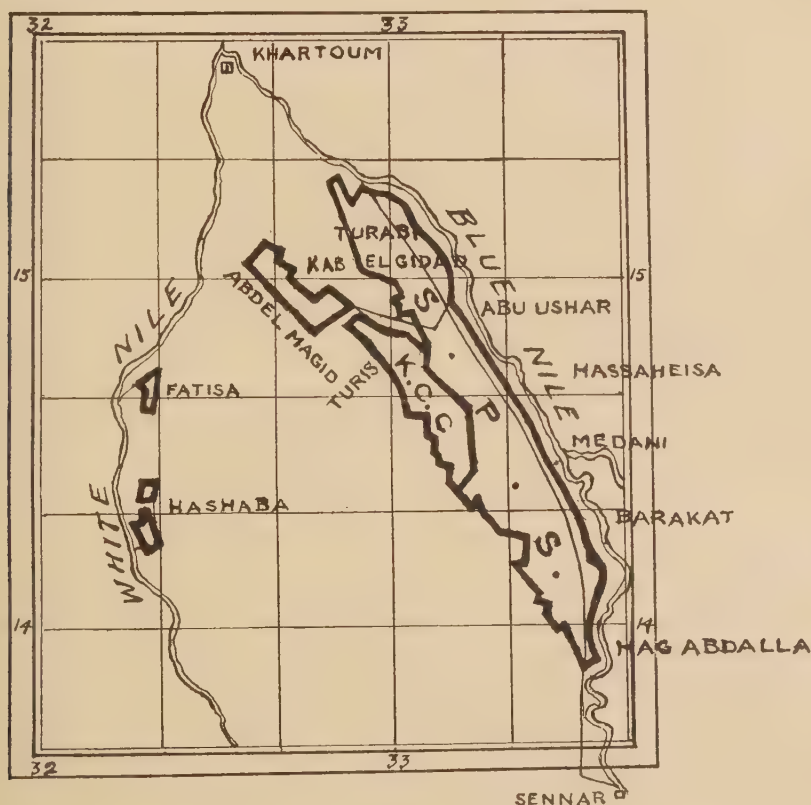
Fig. 1.

feddans* of cotton controlled by the Sudan Plantations Syndicate, and 40,000 by the Kassala Cotton Company are grown yearly in this area. Abdel Magid is one of the Alternative Livelihood Schemes for the Arab population displaced when the Gebel Aulia Dam was built on the White Nile. It lies on the north-west of the Gezira and is irrigated by a feeder canal taking off from the main Gezira canal. At present about 8,000 feddans of cotton are grown annually.

Other Alternative Livelihood Schemes are watered by pumps from the White Nile.

Life-Cycle and Symptoms of Injury.

The life-cycle of the Cotton Jassid and the nature of the damage to the crop have already been described by Cowland (1947) and the following is a brief summary :—

The egg and nymph stages of the Jassid are of short duration, while that of the adult is long. The adult female lays eggs within the tissues of the main veins and petioles of the leaves ; in October the egg to adult stages take 15 days but between November and February the cycle is longer. There are 5 nymphal instars. The generations are not at all clear cut owing to the length of the adult stage and the fact that nymphs of one generation have matured before their parents have completed oviposition.

The parasites and predators of this insect are not very prevalent and it is unlikely that these natural enemies are of much importance.

As the cotton ages, the Jassids migrate to weeds and other crops, where they exist in small numbers during the spring and summer until the following year's crop is sown.

The adults move to newly sown cotton at the end of August almost as soon as the plant is above the ground. At first the infestation is slight, and does not become noticeable until mid-October, but the population increases rapidly, and reaches a peak at the end of November, after which there is a gradual decrease until February when very few individuals are left on the plants.

Damage is first noticed as a yellowing of the marginal leaf tissues followed by reddening and eventual death of the leaf from the periphery inwards. The attack causes premature ageing of the plant, and shedding of the apical buds and bolls.

The relative abundance of Jassids on cotton and the extent of injury to the crop varies from season to season. In " bad " years damage may be more or less widespread, although as a rule it is most severe in the north and west of the Gezira Scheme and in the northern Alternative Livelihood Schemes, and in other seasons the loss of crop from Jassid attack is mostly confined to these areas.

Spraying Experiments.

During the years 1941–44 small-scale field experiments were carried out in which cotton was sprayed with Bordeaux mixture. The results have been described in the paper already quoted. It was found that although the copper spray gave a reasonable control it could not be recommended for use on a large scale owing to the difficulty of preparation in the field. In December 1944 Dr. W. E. Ripper, F.R.E.S., Managing Director of Messrs. Pest Control Ltd., visited the Sudan at the invitation of the Sudan Government to advise on the possibilities of spraying cotton by mechanically drawn sprayers and brought with him various chemicals for trial under local conditions.

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*One Feddan equals 1·038 acres.

Experiment No. 1.

In December 1944 the following sprays were used in the laboratory and in the field :—

1. 2·5 gm. Blitox (copper oxychloride) in 500 cc. water.
2. 5·0 gm. copper sulphate, 5 gm. slaked lime in 500 cc. water.
3. 3·5 cc. of a xylene emulsion concentrate (12·5 per cent. DDT) in 500 cc. water.
4. 5·0 cc. of a xylene emulsion concentrate (12·5 per cent. DDT) in 500 cc. water·

Adult Jassids were enclosed in a laboratory apparatus, sprayed with a regulated dose of the above solutions under standard conditions and then immediately transferred to cotton plants which had previously been enclosed in the type of cage developed by the author. Counts of dead and living insects were taken the following day. It was found that the Blitox and Bordeaux mixture gave no kill but that both concentrations of DDT gave 100 per cent. kill.

These sprays were then applied to two series of cotton plants which were caged when dry and in which each plant was infested with 12 adult Jassids on the following day. One series of plants was examined 2 days after infestation and the second series 20 days after.

The results are given in Table I.

TABLE I.
Jassid counts.

Treatment	Two days after infestation		Twenty days after infestation			Nymphs
	Adults		Adults			
	Live	Dead	Live	Dead	Missing	Live
0·5 per cent. Blitox	12	0	3	2	7	0
Bordeaux Mixture 5 : 5 : 50 ...	10	2	6	2	4	0
0·09 per cent. DDT Emulsion	1	11	0	0	0	0
0·13 per cent. DDT Emulsion	0	12	0	0	0	0
Control	12	0	5	2	5	6

The copper sprays were little better than the controls in killing the adults but no nymphs were found at the second examination in the treated cages while 6 were found in the controls.

Experiment No. 2.

In January 1945 the following sprays were tested :—

1. 2·5 gm. Blitox, and 1 gram Agral 11 in 500 cc. water.
2. 5 gm. copper sulphate, 7·5 grams slaked lime and 1 gm. Agral 11 in 500 cc. water.
3. 3·5 cc. of xylene emulsion (12·5 per cent. DDT) in 500 cc. of water.

Ten pairs of adults were inserted in each cage and examined 20 days later ; one series of tests being carried out on August-sown cotton and the other on cotton sown in October.

TABLE II.

Spray	August-sown cotton			Nymphs	October-sown cotton			Nymphs
	Adults		Missing	Live	Adults		Missing	Live
	Live	Dead			Live	Dead		
Blitox	14	4	2	8	11	3	6	11
Bordeaux	8	5	7	4	5	3	12	15
DDT	0	19	1	0	0	12	8	0
Control	18	2	0	8	7	4	9	54

DDT was then tried out against the nymphs. Plants were sprayed, caged and infested with 20 nymphs per cage. The results after 12 days were as follows :—

DDT—No living nymphs or adults.

Control—16 living adults and 3 living nymphs.

The superiority of DDT over the copper compounds, both against nymphs and adults, is clearly demonstrated from the above experiments. Unfortunately the lateness of the 1944–45 season and the limited quantity of DDT available did not permit experiments to assess the residual toxicity of the DDT spray. The results, however, proved so promising that a large-scale experiment was planned for the 1945–46 cotton crop.

Experiment No. 3 (1945–46 season).

Cotton plants of the variety Domain's Sakel were sprayed, caged and infested on 19th November 1945, and examined 2 days later. The sprays used and the insect counts taken are shown in Table III.

TABLE III.

Spray	No. of adults alive	No. of adults dead	Percentage kill
0·04 per cent. ⎫	4	18	82
0·02 ,, ,, ⎪ Gamma isomer of Benzene Hexa-	19	3	14
0·01 ,, ,, ⎬ chloride	16	9	36
0·005 ,, ,, ⎭	28	2	7
0·1 ,, ,, DDT Emulsion	0	31	100
0·05 ,, ,, ,, ,, ...	0	24	100
0·025 ,, ,, ,, ,, ...	0	25	100
0·012 ,, ,, ,, ,, ...	5	24	83
0·05 ,, ,, ,, ,, ⎫	0	24	100
0·5 ,, ,, Dynone ⎭			
0·1 ,, ,, DDT suspension	24	3	11
0·05 ,, ,, ,, ,, ...	8	18	69
Untreated	27	1	4
,, ...	28	1	3

These figures show that 0·1–0·025 per cent. DDT emulsions gave excellent initial kills, and that the DDT suspension gave erratic results. At the concentrations used the "Gammexane" emulsions were not as effective as those of DDT.

Experiments on the Residual Toxicity of DDT and "Gammexane".

Experiment No. 4.

Using a knapsack sprayer some rows of X1730 cotton were treated with the following sprays on 29th–30th November :—

Emulsions containing 0·1 per cent., 0·05 per cent., 0·025 per cent. DDT.

Suspensions containing 0·1 per cent., 0·05 per cent., 0·025 per cent. DDT.

Emulsions containing 0·1 per cent., 0·075 per cent., 0·05 per cent. gamma isomer BHC.

Each treatment was applied to 2 plants which were then enclosed in a cage. A number of adults and nymphs were released in each cage at intervals of 1, 11, 17, 24 and 37 days after treatment and mortality counts were taken 2 days after infestation. The results are shown in Table IV.

All concentrations of DDT emulsions and "gammexane" gave very good initial kills of adults and nymphs. Suspensions containing 0·1 per cent. and 0·05 per cent. DDT were not very good and 0·025 per cent. DDT was very poor.

In the second count, 11 days after treatment, good kills were still maintained by 0·1 per cent. and 0·05 per cent. DDT emulsions and by solutions containing 0·1 per cent. and 0·075 per cent. gamma isomer BHC.

In the 3rd, 4th and 5th counts the residual toxicity of the sprays had considerably lessened, especially in the lower concentrations, and mortalities decreased as time progressed. The adult mortalities were in all cases higher than in the controls while the nymph mortalities were still fairly good except for 0·025 per cent. DDT emulsion, 0·05 per cent. and 0·025 per cent. DDT suspensions. Of the sprays used the 0·1 per cent. DDT emulsion undoubtedly gave the best initial and residual kills for both adults and nymphs.

Experiment No. 5.

As in Experiment No. 4 more cotton plants were treated on 11th December with 0·6 per cent., 0·4 per cent., and 0·2 per cent. DDT suspensions and were similarly infested 2, 13, and 29 days after treatment. Mortality counts are shown in Table IV. All concentrations gave excellent initial kills of adults and nymphs, and the residual toxicity of the 2 higher concentrations was good, particularly against nymphs. The 0·2 per cent. DDT suspension appears to be similar in initial kill and residual toxicity to the 0·1 per cent. DDT emulsion of the previous experiments.

TABLE IV.

Jassid Counts on Cotton treated with Emulsions and Suspensions of DDT and with Gammexane Emulsions.

Sprayed 29–30 Nov. 1945 with DDT emulsions.

Date Infested	0·1% Adults		0·1% Nymphs		0·5% Adults		0·5% Nymphs		0·025% Adults		0·025% Nymphs		Control Adults		Control Nymphs	
	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.
1–2 Dec.	0	31	0	7	0	49	1	9	0	37	0	11	42	10	63	1
10–11 Dec.	1	40	0	15	6	42	1	17	14	23	0	19	40	11	24	5
17 Dec.	11	39	4	14	15	38	5	14	36	11	7	15	39	12	17	7
24 Dec.	10	49	8	7	20	25	8	7	27	21	13	9	42	11	12	12
6 Jan.	29	19	9	14	34	14	7	8	40	20	33	3	59	9	14	1

Sprayed 29–30 Nov. 1945 with DDT suspension.

Date Infested	0·1% Adults		0·1% Nymphs		0·05% Adults		0·05% Nymphs		0·025% Adults		0·025% Nymphs		Adults		Nymphs	
1–2 Dec.	7	37	0	23	25	34	4	5	43	19	17	5	17	24	9	18
10–11 Dec.	8	58	4	3	14	39	3	10	29	22	2	15	43	7	27	1
17 Dec.	19	24	3	15	19	48	5	8	29	46	8	6	37	11	24	3
24 Dec.	27	36	5	4	13	41	2	11	19	32	13	5	50	6	19	0
6 Jan.	43	19	8	6	29	28	11	13	31	36	8	7	51	13	10	2

Sprayed 29–30 Nov. 1945 with Gammexane emulsion.

Date Infested	0·1% Adults		0·1% Nymphs		0·075% Adults		0·075% Nymphs		0·05% Adults		0·05% Nymphs		Adults		Nymphs	
1–2 Dec.	0	57	0	22	0	39	0	6	0	46	0	12	55	7	66	0
10–11 Dec.	0	44	4	17	1	37	0	22	27	11	5	11	28	8	14	8
17 Dec.	15	39	1	15	10	49	2	15	10	40	1	10	51	6	19	11
24 Dec.	27	46	1	10	3	51	0	17	21	41	0	8	49	9	9	9
6 Jan.	47	22	4	8	41	17	6	8	44	15	3	3	68	9	7	0

Sprayed 11th Dec. 1945 with DDT suspension.

Date Infested	0·6% Adults		0·6% Nymphs		0·4% Adults		0·4% Nymphs		0·2% Adults		0·2% Nymphs		Adults		Nymphs	
13 Dec.	0	45	0	20	0	61	0	19	0	23	0	18	37	5	28	3
24 Dec.	0	58	0	19	0	63	0	9	7	42	4	11	53	2	7	2
7 Jan.	16	42	2	13	12	42	6	11	13	55	9	2	50	5	17	4

A = Alive ; D = Dead.

Field Experiments leading to Large Scale Trials.

Pressure knapsack sprayers working at 75 lb. per square inch were used in all the following experiments.

These experiments were carried out in order to compare the effects of applications of emulsions and suspensions of DDT on the cotton plant. In the Gezira and White Nile Alternative Livelihood Schemes there is a rigid closed season for cotton from the 1st June to the beginning of August. The experiments were therefore started at Shambat, outside these areas, where cotton is grown for experimental purposes in the off-season.

Experiment No. 6.—Phytocidal test.

X1730 cotton, 9 weeks old, was sprayed, on 26th July 1945, with 1·5 per cent., 0·3 per cent., 0·2 per cent., 0·1 per cent. DDT suspension and with 0·5 per cent., 0·2 per cent., 0·1 per cent., 0·05 per cent. DDT emulsion.

These plants were examined 5 and 42 days later. There was no injury to the plants treated with DDT emulsions, but the suspensions from 0·1 per cent. to 1·5 per cent. caused serious leaf distortion. Young leaves became curled and developed parallel venation with almost total suppression of the lamina, as in fig. 2. Plants thus affected received a severe check in growth and did not produce normal leaves for about 3 weeks in the case of those plants treated with 0·1 per cent. DDT suspension and much longer for those treated with higher concentrations.

Fig. 2.—Malformed leaves of Cotton.

Experiment No. 7.—DDT Emulsion against Jassids.

Sakel type cotton 9 weeks old was sprayed, on 18th September 1945, with 0·05 per cent., 0·1 per cent., 0·2 per cent. and 0·5 per cent. DDT emulsion. Table V gives nymph counts taken before and after treatment.

The counts on the control plants, before the treated plants were sprayed, were 73, and 1 day and 10 days after 72 and 21 respectively. At 18 days the counts on old foliage were 30 and new growth 24.

TABLE V.

Nymph counts per 4 plants of 10 leaves.

Treatments	Counts before spraying	Counts after spraying			
		One day	Ten days sprayed and new growth combined	18 days	
				Sprayed foliage	New growth
0·05 per cent. DDT ...	45	0	13	3	9
0·1 ,, ,, ,, ...	69	0	8	0	8
0·2 ,, ,, ,, ...	99	0	6	0	2
0·5 ,, ,, ,, ...	78	0	8	0	6

The initial kill was excellent at all concentrations and the residual toxicity on sprayed foliage good. No phytocidal injury was visible.

Experiment No. 8.—Phytocidal tests on seedling cotton.

These tests were carried out on the Gezira Research Farm, Wad Medani on a plot of X1730 cotton which was sown on the 27th August.

The following concentrations of DDT emulsion were used in each case : 0·025 per cent., 0·1 per cent., 0·15 per cent. DDT.

(*a*) Sprayed 16th September ; plants 4–6 inches in height, in 3rd leaf stage. Distortion and parallel venation was evident about a week after spraying; its severity increasing as the concentrations increased and in the highest concentrations a few plants were killed.

(*b*) Sprayed 23rd September at 13.00 hours. Plants 7–9 inches in height, in 4th leaf stage. Examined seven days later. On the two lowest concentrations some distortion was beginning to show on a few plants but none was apparent on the plants sprayed with the two higher concentrations.

(*c*) Sprayed on 26th September at 08.00 hours and 14.00 hours. Plants 8–9 inches in height, in the 5th–6th leaf stage. Two re-sown plants and one older plant sprayed at 08·00 hours with 0.025 per cent. emulsion showed distortion : while one plant sprayed at 14.00 hours with 0.025 per cent. emulsion and one with 0·15 per cent. emulsion were distorted.

(*d*) Sprayed on 29th September at 13.30 hours. One plant sprayed with 0·1 per cent. emulsion and one sprayed with water alone were distorted.

(*e*) Sprayed on 30th September at 13.30 hours. No injury resulted.

(*f*) Sprayed 6th October. No injury resulted.

These tests show that leaf distortion or injury following an application of DDT emulsion, at the strengths used above, only occurs on very young cotton. Some of the distortions noticed above were undoubtedly due to the use of spray lances which were dirty from an earlier application as is shown from the fact that one plant was distorted after being sprayed with water.

The water used in these experiments was very alkaline (*p*H 9) and this caused a small amount of DDT to be precipitated in the spray liquid. Some of the oil from the DDT emulsion in xylene was inclined to float on the surface of the spray in the absence of agitation.

Field Experiments with Power Sprayer.

A trailer power sprayer drawn by a tractor (Pl. II, figs. 1 and 2) was used for large-scale experiments. The spray was applied at the rate of 100 gallons per feddan

at a pressure of 250 lb. per square inch. Short and long nozzle arms (Pl. II, fig. 3) were used alternatively to give complete cover of the foliage on all sides. The tractor and sprayer wheel distances were adjusted to 160 cm. to correspond with the standard cotton ridge width of 80 cm.

Experiment No. 9. DDT Emulsion and Copper Oxychloride.

At the Gezira Research Farm a 5-feddan plot of X1730 cotton was sprayed with 0·1 per cent. DDT emulsion on 16th October 1945, a further 5-feddan plot was sprayed with Blitox (copper oxychloride) at 5 lb. per 100 gallons, while a third 5-feddan plot was used as control.

All the cotton was sown at approximately the same time, *i.e.*, 15th to the end of August. Counts of Jassid were made as follows :—

Adults. 1 net consisting of 12 double sweeps per angia (the area between irrigation water channels, in this case ¼ feddan).

Nymphs. 5 plant holes (10 leaves per hole) in each angia. These counts do not show any relation between adult and nymph populations as the method of obtaining each was necessarily different.

TABLE VI.

Jassid Counts.

Date	0·1 per cent. DDT Emulsion		0·5 per cent. Blitox		Control	
	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
15 Oct.	141	321	227	586	Not counted	
18 Oct.	10	66	646	216	Not counted	
29 Oct.	22	30	197	610	202	557
10 Nov.	222	38	1,009	899	1,283	2,002
24 Nov.	599	1,016	437	1,957	890	1,933
8 Dec.	609	1,284	1,045	1,516	1,353	1,595
22 Dec.	490	710	896	872	1,098	722
5 Jan.	485	117	542	98	457	91
19 Jan.	216	33	282	23	223	18
4 Feb.	72	0	93	2	73	4
18 Feb.	3	0	1	0	16	0

The DDT spray gave an immediate good control of adults and nymphs and practically no increase in numbers occurred for a month. The Jassid population were again fairly high 40 days after spraying though less than on the control plot. " Hopper-burn " was very evident on the unsprayed plot at that time but still scarcely present on the treated plot.

The copper oxychloride had not decreased the Jassid population the day after treatment but there was a decrease 12 days later. The population remained only slightly below that of the untreated plot for the rest of the season.

Experiment No. 10.—Comparison of DDT suspension with combined spray of DDT emulsion and nitrate of soda.

The DDT suspension used here had much finer particles than that which caused leaf distortion in Experiment No. 6.

Spraying was carried out on 27th November 1945, the treatments being :—

1. 0·1 per cent. DDT emulsion, 0·5 per cent. nitrate of soda—with 8 oz. of Agral per 100 gallons of spray.

2. 0·1 per cent. DDT suspension.

TABLE VII.

Jassid Counts.

Date	DDT + Na No. 3		DDT Suspension		Control	
	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
29 Nov.	9	4	106	158	818	974
14 Dec.	338	50	284	28	2,140	908
27 Dec.	445	42	202	8	1,218	316
10 Jan.	370	20	226	4	518	52
24 Jan.	136	3	68	0	46	2
4 Feb.	108	0	12	0	22	0

The control obtained by this combined spray seems to be as good as that by 0·1 per cent. DDT emulsion. Reinfestation by adults was earlier, probably on account of the greater movement of Jassids from other plots at that time of the year following the beginning of the maturation of the earlier sown cotton. The nymphal counts, however, remained low through the season. This plot was decidedly greener, and kept growing for a longer period than usual, but to what, if any extent, the prolonged growth was due to nitrate of soda is not known.

The DDT suspension gave fairly good control though the initial control was not quite as good as with a 0·1 per cent. emulsion.

Power Spraying Trials in the Gezira and at Abdel Magid.

Experiment No. 11.—Spraying at Kab el Gidad (Northern Gezira).

At Kab el Gidad an area of 1,000 feddans was chosen for treatment, with a similar area for control.

A Standard cotton number (fig. 3) here consists of 9 tenancies each of which contains 10 feddans. Each number has an irrigation channel (Abu Ishreen) which is fed with water from a canal. Each tenancy (hosha) has a field channel (Abu Sitta) which gets it water supply from the Abu Ishreen. The Abu Sitta supplies water to the watering ditches (gadwells) within the crop, whence it goes between the ridges. Banks (tagnets), alternating with the gadwells, are used to hold the water in the furrows. The area between a gadwell and tagnet is an angia of which there are 16 in a hosha. There are approximately 187 ridges in a hosha.
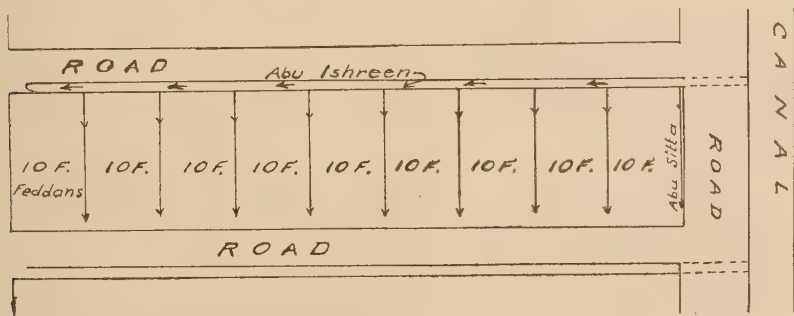

Fig. 3.

At the time when spraying is undertaken the cotton requires an abundant supply of water. In consequence the intervals between watering are reduced to 11–12 days, and the ground seldom gets dry during these intervals. This makes operations by a

large power sprayer difficult and, as a result, water must sometimes be withheld for a further 1–2 days.

The variety of cotton grown in Kab el Gidad is Domains Sakel. The height over a period of years has been as follows :—

TABLE VIII.

Average height of Sakel Cotton in centimetres.

Date	39–40	40–41	41–42	42–43	43–44	44–45	Average cm.	inches
End Sept.	46·2	52·6	51·5	45·7	47·3	52·4	49·3	19·4
End Oct.	73·4	86·5	89·7	85·4	86·1	88·3	84·9	33·5
End Nov.	74·0	90·2	90·5	94·7	100·0	96·5	91·0	35·9
End Dec.	77·5	93·1	92·9	92·8	100·4	97·1	92·3	36·4

The tractor and sprayer had only 27 ins. clearance from the ground but in spite of this it was able to spray cotton much taller than this. The cotton was bent over by the machine but became erect again after it had passed. In mid-November it was able to treat successfully a crop with an average height of four feet. At this time the bolls were beginning to set, and the few knocked off by the machine were negligible. Depending on the height of the crop, the spray booms were easily raised or lowered to give the optimum coverage. Damage caused by the machine in turning was negligible.

Two sprays were used :—

(a) Blitox at 5 lb. per feddan. Size at ½ lb. per 100 gallons of spray was used as a sticker.

(b) DDT emulsion usually at 0·1 per cent. DDT, but some tenancies were treated with half this concentration.

Before the experiment started pairs of neighbouring numbers having approximately the same sowing dates were selected. From each pair the number to be sprayed was chosen at random for comparison with its untreated neighbour. An endeavour was made to follow this pre-arranged programme, but some deviation had to be made owing to the water requirements. In such cases the sprayed and untreated numbers were reversed.

As far as possible Jassid counts were made before and after treatment on sprayed and control numbers. Since spraying was sometimes carried out the day before watering it was impossible to get all the post-spraying counts desired. When a number was being sprayed its neighbouring control number was usually undergoing irrigation, thus making it impossible to obtain Jassid counts for each pair on the same day.

The Jassid counts consisted of one net for adults and 50 (5×10) leaves for nymphs in each angia, making a total in each tenancy of 16 nets for adults and 800 leaves for nymphs. At the end of October the number of adults thus counted on a considerable number of tenancies varied between 450–1,600 and of nymphs between 600–1,300. These figures increased on the unsprayed numbers to reach a maximum of 2,200 nymphs by the end of November and a maximum of 3,200 adults by the middle of December.

In the tenancies sprayed with Blitox the Jassid population was only slightly reduced in most cases after spraying ; while in all cases the population on sprayed numbers at the end of December was much less than on the controls.

Since the initial experiments showed that 0·1 per cent. DDT emulsion gave good control, it was decided to use this concentration for most of the work done by the power sprayer in order to obtain information on residual toxicity.   A 0·05 per cent. DDT emulsion was used on 6 tenancies.   In all cases the DDT sprays gave an excellent kill within 24 hours, reducing both adult and nymphal populations to almost nil.   Numbers treated with 0·1 per cent. DDT continued to be practically free from Jassids for about 3 weeks, due to the good initial kill and to the residual toxicity of the spray.   The populations began to rise slightly after 3 weeks, but they soon began to decline again naturally after the end of November.   The DDT sprays were applied between the 1st and 10th November, except for one tenancy which was treated on 20th October.   On tenancies treated with the 0·05 per cent. spray, the Jassid counts at the end of December were only slightly higher than those treated with double this strength.   From this it would seem that the 0·05 per cent. DDT spray is almost as effective as the 0·1 per cent. DDT spray when applied towards the end of the spraying season.

Observations during experiment.

The Jassid infestation for the 1945–46 season was of average intensity for recent years.   When the experiment started Jassid populations were high but no injury was visible.   During the first week of November the first symptoms of injury were noticed and " hopperburn " gradually became worse as the season progressed. The plants treated with DDT kept their healthy green appearance throughout the season and " hopperburn " was negligible.   Because of the longer growing period thus permitted, they produced more flowers and bolls than untreated cotton.   Plants treated with Blitox suffered almost as much from " hopperburn " as untreated cotton.

The following cotton insects were also killed by the DDT sprays :—*Campyloma nicolasi*, R. & P., *Podagrica puncticollis*, Wse., *Bemisia gossypiperda*, M. & L., and *Pyrgomorpha* sp. Adult whiteflies (*Bemisia*) were killed but the pupae were not affected.

Yields.

In each tenancy the grades and yields of seed cotton were taken at the end of the season.   An analysis of these yields is given in Table IX.   These figures show the success obtained by spraying with DDT at both concentrations, and the comparatively poor results given by treatment with copper oxychloride (Blitox).   Owing to the fact that only 60 feddans were treated with 0·05 per cent. DDT emulsion towards the end of the spraying season it does not necessarily follow that this concentration would be as good as 0·1 per cent. DDT emulsion if used earlier in the season.

The grades of cotton picked deteriorate progressively as the picking season advances.   During the first three months of picking, *i.e.*, January to March, the yield increases due to treatment with DDT were not very large, but in the final pickings of April and May considerable increases were obtained, with a larger proportion in grade 5.   This agrees with the fact that treated cotton has a longer growing season and produces more flowers and bolls than untreated cotton.   The larger increases in grade 5 than in grade 6 may be due to the treatment having the effect of raising the quality of the cotton.

Increases in yield obtained by treatment with Blitox were slight and in the lowest grades.

Experiment No. 12.—Power spraying at Abdel Magid.

Here 200 feddans of X1730 cotton were treated with 0·1 per cent. DDT emulsion between 11th and 18th November.   Jassid counts were not carried out as in the previous experiment, but occasional counts showed that the Jassids were more numerous than in Kab el Gidad.   This high Jassid population had already caused considerable " hopperburn " on the lower leaves.

TABLE IX.

Analysis of yields in Kab el Gidad Block 1945/46.

Spraying Treatment	Area in feddans	Average Yield in each Grade						Average Yield of cotton in k.p.f.	Increase in Yield k.p.f.	Per cent. Increase of Total Yield over	
		1	2	3	4	5	6			Local Control	General Control
0·1 per cent. DDT Emulsion	540	0·04	0·09	0·65	0·67	2·16	1·88	5·49	1·49	37·2	38·6
Local Control ...	530	0·02	0·12	0·35	0·77	1·36	1·38	4·00	—	—	—
0·05 per cent. DDT Emulsion	60	0·10	0·15	0·65	0·64	2·32	2·29	6·15	1·95	46·4	55·3
Local Control... ...	60	—	0·06	0·12	0·60	1·32	2·10	4·20	—	—	—
Blitox 0·5 per cent. ...	400	0·04	0·07	0·44	0·52	1·63	1·57	4·27	0·38	9·8	7·8
Local Control	400	0·02	0·12	0·32	0·84	1·39	1·20	3·89	—	—	—
General Control ...	990	0·03	0·09	0·35	0·65	1·45	1·39	3·96	—	—	—
Average of local controls ...	—	—	—	—	—	—	—	—	—	—	—

k.p.f. = kantars per feddan

Soon after spraying the cotton appeared much healthier and had a larger number of flowers than untreated cotton. About one month after treatment the benefit was so marked that the treated tenancies could be easily picked out from a distance.

Yield figures were taken at the end of the picking season, but the cotton was not divided into grades. An analysis of the yields is shown in Table X.

TABLE X.

Analysis of yields at Abdel Magid 1945/46.

Spraying Treatment	Areas in feddans	Average yield of cotton in k.p.f.	Increase in yield k.p.f.	Increase of total yield over control
0·1 per cent. DDT emulsion	200	3·96	0·76	23·75
Control	272	3·20	—	—

Owing to the nomadic habits of the tenants in this area a considerable amount of cotton was left unpicked and fell on the ground, particularly on treated tenancies, but in spite of this the latter yielded 0·76 k.p.f. (kantars per feddan) more than the controls.

Experiment No. 13.—Power spraying in Turis.

In this area 450 feddans of Sakel cotton were treated with 0·1 per cent. DDT emulsion between 20th and 25th November. At this time the Jassid population was nearing its peak, and injury was already severe on the lower parts of the plant.

In all numbers where treatment was carried out at least two tenancies were left untreated as controls.

At the beginning of January the treated cotton showed much less " hopperburn " than the controls, but matured almost as quickly due to exhaustion of the soil nutrients.

An analysis of the yields is given in Table XI. The yield increases, chiefly in the lower grades, were not so large as in the previous experiment. This is due to a combination of late spraying and soil poverty.

TABLE XI.

Analysis of yields in Turis Block 1945/46.

Spraying Treatment	Area in feddans	Average yield in each grade						Average yield of cotton in k.p.f.	Increase in yield k.p.f.	Increase of total yield over control
		1	2	3	4	5	6			
0·1 per cent. DDT emulsion	450	0·32	0·26	0·66	0·87	0·99	0·27	3·37	0·42	14·24
Control ...	160	0·33	0·31	0·50	0·74	0·88	0·19	2·95	—	—

Summary.

A short account of the life-cycle and symptoms of damage of the cotton Jassid, *Empoasca lybica*, de Berg., is given. In the initial cage experiments it is shown that DDT emulsion was superior to the copper compounds used. Experiments on the residual toxicity of DDT and " Gammexane " sprays are described. Tests with DDT emulsion showed that very young cotton is injured by emulsion containing as little as 0·025 per cent. DDT, but that emulsions containing up to 0·5 per cent. DDT can be used with safety on plants which are two months old. A coarse DDT

suspension caused severe leaf distortion, but a fine suspension used later was quite safe at the concentration used.

Field experiments with a power sprayer using various sprays were carried out at Gezira Research Farm. These were followed by large-scale spraying trials on 1,000 feddans at Kab el Gidad, 200 feddans at Abdel Magid, and 450 feddans in Turis. The sprays applied were copper oxychloride (Blitox) and DDT emulsion. Jassid counts were taken before and at intervals after treatment on the Gezira Research Farm and at Kab el Gidad. Copper oxychloride did not give good control of Jassids, but 0·1 per cent. and 0·05 per cent. DDT emulsion gave excellent kills and remained toxic to Jassids for 2–3 weeks.

The benefit of spraying with DDT emulsion is amply demonstrated by the increased yield obtained on treated areas at Kab el Gidad, Abdel Magid and Turis.

Acknowledgements.

Reference.

COWLAND, J. W. (1947). The Cotton Jassid (*Empoasca libyca*, Berg.) in the Anglo-Egyptian Sudan and experiments on its control.—Bull. ent. Res., **38,** pp. 99–115.

Field experiments with a Trailer Power Sprayer drawn by a tractor.

THE PARASITES AND PREDATORS OF POTATO APHIDS.

By J. A. DUNN, M.Sc.
*School of Agriculture, Cambridge.**

The survey of the potato Aphids in the Northern Agricultural Advisory Province, started by F. H. Jacob (1944) in 1942, was continued throughout 1944 and 1945, chiefly in Northumberland, by J. P. Rogerson and the author. In addition, the latter worker accumulated information on the natural enemies which affect the increase of the Aphid populations ; this was limited in 1944 to data on the parasites but broadened in 1945 to incorporate the predators. The aim was to find out what, and when, insect enemies helped in the control of their hosts, and to what extent. More detailed knowledge of these biological factors was obtained from a small plot of potatoes grown in a suburban garden, where weekly counts were made.

TECHNIQUE.

In the field, Aphid populations were estimated by means of the " 100 leaves system " devised by Davies (1934). All parasitised or unhealthy looking Aphids found on the leaves thus counted, were collected, together with predatory material (in 1945) in the form of eggs, larvae, etc., and taken back to the laboratory for hatching and rearing. Most field centres were visited twice in a season and some three times. Potato plants were examined and all relevant insect life noted, with as much care, and at the same time with as little disturbance, as possible. All parasitised Aphids from the plot were collected and after the date of hatching and identity had been confirmed, the issuing parasites were liberated as closely as possible on to the same leaves as those from which their cocoons had been taken. A similar procedure was used with the pupae of predators, though here of course the same care over the liberation of the adults was not necessary.

The parasitised material from both field and plot collections was segregated into numbered vials, each individual cocoon having a small piece of the original potato leaf adhering to it ; this was often a disc-shaped piece cut out by placing the tube over the cocoon on the leaf and rotating with a little pressure. A piece of leaf of this size provided, in most cases, enough moisture to prevent the parasite pupa from drying out, and not sufficient to give rise to mould formation.

The eggs and larvae of predators, collected during the field counts, were put individually into a numbered series of larger tubes (3 in. × 1 in.) and life-history records kept. These predators were reared on *Macrosiphum pisi*, Kalt., a constant and plentiful supply of which was obtained from colonies raised on potted beans.

THE SPECIES OF APHIDS UNDER CONSIDERATION.

Though other species of virus-transmitting Aphids occur on potato, the only ones in the Northern Province taken into account by Jacob were *Myzus persicae*, Sulz., *Aulacorthum solani*, Kalt., *Macrosiphum solanifolii*, Ashm., and *Doralis rhamni*, Boyer. Those considered in the present paper are the first three ; *D. rhamni* occurred to such a restricted extent in the fields visited in 1944 and 1945 that it does not merit inclusion. Theobald (1926) gives descriptions, with drawings, of these three species and Davies (1934), descriptions and photographs for their recognition and distinction during actual field work.

Myzus persicae, Sulzer.

This is generally recognised as the most important vector of potato virus diseases and the list of 26 different plant viruses transmitted by this species (Bawden, 1943) includes seven affecting the potato.

G

(a) Parasites.

The amount of attention devoted to this Aphid is reflected by numerous and world-wide records.

BRACONIDAE (APHIDIINAE).

Aphidius matricariae, Hal.	...	...	...	...	England
Aphidius sp. (probably *cardui*)	...	...	...	...	France
Aphidius sp.	...	...	...	...	...	Italy
Aphidius nigriteleus, Smith	...	...	...	...	U.S.A.
Aphidius persicae, Froggatt	...	...	...	...	Australia
Aphidius phorodontis, Ashm.	...	...	...	...	Canada and U.S.A.
Diaeretus rapae, Curt.	...	...	...	...	U.S.A. and Hawaii
Ephedrus incompletus, Prov.	...	...	...	...	U.S.A.
Ephedrus nitidus, Gah.	...	...	...	...	Canada
Lysiphlebus sp.	...	...	...	...	South Africa
Praon simulans, Prov.	...	...	...	...	U.S.A.

In addition to *A. matricariae*, Hal., from England, *A. avenae*, Hal., and *Praon volucre*, Hal., can now be recorded.

APHELINIDAE.

Aphelinus jucundus, Gah.	...	...	...	...	Canada
Aphelinus mali, Hal.	...	...	...	...	Argentina
Aphelinus marlatti, Ashm.	...	...	...	...	Canada
Aphelinus semiflavus, How.	...	...	...	...	Spain and U.S.A.

Records from Australia also give the Cynipids, *Charips aphidae*, Frogg., and *Alloxysta* sp. and the Proctotrupid, *Lygocerus niger*, How., as parasites of *M. persicae* but these most probably are hyperparasites through *Aphidius* spp.

(b) Predators.

Most of the forms known to feed on other Aphids are doubtless also capable of preying on *Myzus persicae* and vice-versa in view of the more generalised feeding habits of Aphid predators. Therefore any attempt to list the recorded predators seems unnecessary beyond saying that with the exception of one Indian record of Chamaemyiid, *Leucopis griseola*, Fall., which it is stated is also a general feeder, all recorded predators of *M. persicae* are Chrysopids, Coccinellids or Syrphids. It is proposed to deal with those found during the present survey (all of which fed on potato Aphids indiscriminately) later in the paper.

Macrosiphum solanifolii, Ashmead.

This is the largest of the three species as well as the most active. It shows a greater preference towards colonising the flower heads and upper parts of the potato plant than the other two, and when disturbed falls readily to the ground. Bawden lists four potato virus diseases of which *M. solanifolii* can act as vector.

(a) Parasites.

BRACONIDAE (APHIDIINAE).

Aphidius matricariae, Hal.	...	...	...	...	England
Aphidius polygonaphis, Fitch	...	...	...	...	U.S.A.
Aphidius rosae, Hal.	...	...	...	...	U.S.A.
Diaeretus rapae, Curt.	...	...	...	...	U.S.A.
Praon aguti, Smith	...	...	...	...	U.S.A.

A. avenae, Hal., *A. ervi*, Hal., and *P. volucre*, Hal., were hatched during this survey.

APHELINIDAE.

Aphelinus jucundus, Gah.   ...   .   U.S.A.

(*b*) *Predators.*

The sole reference found to predators was in a paper by Dicker (1940) dealing with the " Rubus Aphides " (which include *Macrosiphum solanifolii*). This author stated that the larvae of *Coccinella septempunctata*, L., and *Adalia bipunctata*, L., were present from April until the end of July, Syrphids and Neuroptera from April until autumn in diminishing numbers, and that meanwhile predacious Hemiptera like *Anthocoris nemorum*, L., *A. nemoralis*, F., *Orius majusculus*, Reut., *O. minutus*, L., and Capsids were active, remaining until October.

Aulacorthum solani, Kaltenbach.

This species is much less important and is seldom present in potato fields in numbers approaching those of the two Aphids dealt with above. It is also a less effective virus transmitter.

(*a*) *Parasites.*

As in the case of the two previous Aphids, *Aphidius matricariae*, Hal., is recorded from England ; there are no other records. During the present work, *A. avenae*, Hal., *A. ervi*, Hal., and *Praon volucre*, Hal., found parasitising *Macrosiphum solanifolii* were hatched in addition.

(*b*) *Predators.*

No reference could be found to any predators.

THE PARASITES.

The primary parasites were BRACONIDAE (APHIDIINAE), but all the parasites belonged to the Hymenoptera. All the known species of APHIDIINAE are solitary internal parasites, and of the two genera, *Aphidius* and *Praon*, hatched during the survey, the former predominates, both in the number of species the genus contains and in the extent to which certain of these species occur.

Limited time made it impossible to follow in detail the immature stages of these parasites but work carried out by Vevai (1942) on *Aphidius matricariae*, and by others on various species, shows that the bionomics of each species display great similarity, and these together with personal observations can be generalised.

An Aphid parasitised by *Aphidius* sp. is a familiar object in the later stages of parasitisation, when its dry, swollen and distorted, parchment coloured skin is seen adhering to the host plant. Normally apterous viviparous forms are selected for oviposition, though parasitised alates are found. There is usually one egg per host, deposited invariably in the abdomen, but as many as ten have been known to hatch within one host ; only one parasite per Aphid completes development. A half-grown Aphid is the usual victim and those parasitised in or before the third instar fail to reproduce ; an Aphid in a later stage reproduces until the parasite larva reaches its third instar. The vital organs of the host are attacked when the parasite enters its fourth-larval stage, which is an active one ; it is then that the Aphid shows obvious, outward signs of parasitisation, assuming a darker, shining appearance with the parasite larva visible through the cuticle. The parasite can be seen rotating several times as it consumes all the remaining food within reach. Before the host's legs dry and lose their hold, the *Aphidius* larva cuts a ventral slit through the cuticle and attaches it to the food-plant by part of a silken cocoon, which it commences spinning inside the drying Aphid skin. The adult *Aphidius* emerges through a small circular hole that it cuts generally through the dorsum of its host in the region of the cornicles.

Praon is the only genus to exhibit any variation. Here a larva leaves its host just before pupating and constructs a tent-like outer wall of white silk, below the dead Aphid, surrounding an inner cocoon. The emerging adult cuts a hole through the double wall, often anteriorly beneath the host's rostrum.

The Aphidiines are described under the Flexiliventres in Marshall's work on British Braconidae (1899).

Aphidius avenae, Haliday.

In the adult (fig. 1) the characteristic of strong and distinct wing nervures given by Marshall is fairly constant, but individuals exist in which nervures (and pterostigma) are very faint. Time of death seems also to affect this character, and maximum intensity of neuration does not appear to be reached until several hours after hatching. This applies to APHIDIINAE in general in which the wing veins of the imago, killed shortly after emergence, are often found to be indistinct. The usual number of antennal joints in the females hatched was 17 ; 18 and 16 occurred much less frequently. The majority of male antennae had 20 joints, whilst a few had 21 and 19 and an individual with 22 and another with only 18 were obtained. At times the last two joints were fused together.

Fig. 1.—*Aphidius avenae*, Haliday. Size 2·7 × 4·5 mm. (ex *Myzus persicae*).

A. avenae can be regarded as the only parasite to exercise a controlling influence on potato Aphid populations in the Northern seed-growing areas for it occurred to the complete exclusion of the other three primary parasites, except on lowland farms (generally near market gardens) and even here it was easily the most numerous.

It has long been known to be a polyphagous species and although it parasitised *Myzus persicae* to a somewhat lesser degree than the other two potato-infesting Aphids, host discrimination was not very apparent. This, of course, could be

expected, as all species of Aphids feeding on the same plant should, with only slight specific variation, contain the same plant juices and be of similar chemical make-up. Thus the only difference which could logically determine the choice of host is a physical difference, that of size, linked with the ability of the parasite to overcome this discrepancy. Within limits these polyphagous parasites are quite adaptive, and the size of the host naturally predetermines the size of the ensuing adult. *A. avenae* females hatched from *Myzus persicae* (the smallest of the 3 host species) measured $2 \cdot 7 \times 4 \cdot 5$ mm. and those from *Macrosiphum solanifolii* (the largest) $3 \cdot 1 \times 5 \cdot 2$ mm. Recorded alternative hosts are :—

Amphorophora rubi, Kalt.	...	...	...	...	Britain
Aphis infuscatus, Koch ...	...	...	...	...	Russia
Aphis scabiosae, Schr. ...	...	...	...	...	Britain
Brachycaudus helichrysi, v. d. G.		...	...	...	Britain
Hyadaphis xylostei, Schr.	...	...	...	...	Britain
Macrosiphum granarium, Kirby		...	...	...	Canada
Macrosiphum urticae, Kalt.	...	...	...	...	Britain
Ovatus crataegarius, Walk.	...	...	...	...	Britain
Rhopalosiphum padi, L. ...	...	....	...	...	Britain and Russia

Three new British hosts may be recorded :—

Macrosiphum pisi, Kalt., *M. rubiellum*, Theo., and *M. granarium*, Kirby.

The presence of a host does not cause the same degree of excitement in the female *A. avenae* that it does in *A. matricariae*. When ovipositing, the characteristic attitude is adopted of balancing on the middle and hind legs and drawing the abdomen between them with a sharp forward thrust.

The life-cycle during summer occupies approximately 3 weeks, and parasitised Aphids make their appearance on potato leaves about mid-July. Two generations seem likely under field conditions, with one or two before July on different hosts, or in the case of the same host on different food plants. The sex ratio, $1 \cdot 2 : 1$, showed a slight preponderance of males.

The maximum number of adults emerged five to six days after the cocoons had been collected. All *A. avenae* hatched before the end of September, and it is thought probable that a few adults might successfully pass the winter in grass tufts, straw, etc.

The most appropriate means of hibernation would appear to be as mature resting larvae within cocoons formed from Aphid hosts that had been attacked during the autumn. These cocoons may often be found during winter in crevices, and within the dry and curled-up fallen leaves of the host plant. Parasitised *Amphorophora rubi* on blackberry leaves were found to provide just such winter quarters for *A. avenae*. The cocoons, which are tough and resistant, are capable of withstanding normal winter conditions ; the warmer spring days of the year following bring about the pupation and emergence of the parasites. All immature stages were found to be present in *Myzus persicae* and *Macrosiphum pisi* feeding on clover plants in an unheated glasshouse at King's College, Newcastle, on 5th April, 1945 : these had evidently wintered as apterae, and the first imagines hatched two days later. It is therefore conceivable that generations of *A. avenae* can be produced intermittently throughout a mild winter in unheated greenhouses through Aphid hosts, which are themselves slowly reproducing. Under heated greenhouse conditions, this suggested over-wintering method is made infinitely more possible.

Aphidius ervi, Haliday.

The adult is very similar to the preceding species but has obviously longer antennae, with an extra joint or two. The legs were flavescent in the females, which

was the only sex hatched, and antennae had 18 or 19 joints. It is stated by Marshall to be as common as *A. avenae*, often attacking the same species of Aphids. This was not the case with potato Aphids from which it was hatched on only one occasion in the field. The garden plot yielded more, but here also it was of little importance as a check.

Recorded alternative hosts are :—

Amphorophora rubi, Kalt.	...	...	...	...	England
Aphis gossypi, Glov.	...	...	...	...	Morocco
Aphis scabiosae, Kalt.	...	...	...	...	England
Macrosiphum cyparissae, Koch, *M. picridis*, F., and					
M. pisi, Kalt. ...	...	...	...	...	Jugoslavia
Macrosiphum urticae, Kalt., and *M. rosae*, Kalt.			...		England

The five species of *Macrosiphum*, to which can be added *M. rubiellum*, Theo., seem to indicate a preference for the larger type of Aphid. It was never hatched from *Myzus persicae*.

The life history appears to be very similar to that of *A. avenae*, except that it seldom occurs on a field scale.

Aphidius matricariae, Haliday.

The adult is smaller on the average than the other two species. Male antennae have 16 or 17 joints and female 14 ; females were sometimes hatched with 15, and more rarely, with 13 joints.

Smith (1931) dealing with *Myzus persicae* indicates that under glasshouse conditions a high degree of control is exerted by what is probably *A. matricariae*. The experience of the present writer tends to bear this out. Only in two lowland areas in close proximity to market gardens was this species found.

In the garden plot this was the most important primary parasite but it confined its attacks to *Myzus persicae* and *Aulacorthum solani*, although *Macrosiphum solanifolii* were in the majority. In the field it was only hatched from *Myzus persicae*.

Vevai (1942), who gives drawings and descriptions of the immature stages, found that the progeny of a single mated female confined under a glass chimney with 25 *M. persicae* (apterous viviparous females) on tulip, eradicated the colony within 11–12 weeks, involving approximately 4 parasite generations ; 25 *M. persicae* in a control experiment produced more than 350 progeny at the end of 5 weeks and " countless " numbers at the end of 11.

The oviposition instinct seems highly developed in *A. matricariae* females and they become very excited on sensing a host, whether mated or not. They even take practice oviposition thrusts at cast Aphid skins, leaf-veins and sometimes at nothing.

The sex ratio was nearly 1 : 1, with a slight male predominance. Marshall gives *Brachycaudus helichrysi* as an alternative host and this was also found during the present work, together with *Myzus ornatus*, Laing. Vevai gives *Aulacorthum solani* and *Myzus circumflexus*, Buckt., as other hosts.

Praon volucre, Haliday.

In addition to its peculiar cocoon, the venation of the forewing serves to distinguish the genus *Praon*.

Beirne (1942) describes its bionomics on *Hyalopterus arundinis*, F., and Marshall states that it is the commonest of the *Praon* species as well as being polyphagous ; the latter worker also supplies an alternative host list of 5 known and 2 unknown species of Aphids.

It was only found once in the field on *Myzus persicae* but the garden plot and other potato plants showed that all three species of Aphids could be attacked. It was also found parasitising *Amphorophora rubi* on bramble.

The cocoons of *P. volucre* seem more sensitive to drying out than those of *Aphidius* spp. and several confined in vials in the same manner as the latter, failed to hatch for this reason. Doubtless the type of web cocoon found in *Praon* makes it dependent to a greater extent upon more intimate contact with the surrounding micro-climate of the host-plant leaf than the pupae of *Aphidius* spp., protected as they are by both their own web cocoons and the cuticular covering of their hosts.

The Importance of Primary Parasites.

Jacob (1944) states that the number of Aphids he found parasitised during the survey in 1942 and 1943 was small. The percentage figures given below for the surveys of 1944 and 1945 show that parasitism was slightly greater in the former year, but that it was not high in either year.

	1944	1945
Myzus persicae	13·1	8·9
Macrosiphum solanifolii ...	15·7	9·4
Aulacorthum solani	15·4	12·5*

Parasite attack began about a month after the Aphid infestation had become established. At one centre only (a lowland one) was parasitism obviously exerting control. The first count here was made on 1st August, when 50 per cent. of the apterous *Macrosiphum solanifolii* and 46 per cent. of the *Myzus persicae* were found parasitised. A month later the number of parasitised apterae had risen to over 90 per cent. in both species ; this was despite the fact the crop was late sown, the haulms still a very fresh green and the weather mild, all of which are factors favourable to a high Aphid population.

HYPERPARASITES.

Those found in the present survey were :—

PTEROMALIDAE.	*Asaphes vulgaris*, Wlk.
	Coruna clavata, Wlk.
CERAPHRONIDAE.	*Lygocerus testaceimanus*, Kieff.
	Lygocerus sp. A.
	Lygocerus sp. B.
CHARIPIDAE.	*Alloxysta* sp.
	Charips curvicornis, Cam.
	Charips tscheki, Giraud
	Charips victrix var. *infuscatus*, Kieff.

The above are secondary parasites of the potato Aphids through the primary Aphidiine species dealt with above. They attack these Braconids whilst the latter are still immature within their Aphid hosts, feeding either externally upon their victims (from inside the cocoon) as ectoparasites, or internally as endoparasites. They pupate within the cocoons made by their hosts, and the adults emerge through jagged holes cut through the dorsal cuticle of the dead Aphids ; there are no lids to these holes in contrast to those of the primary Braconid parasites.

Haviland (1920–1922) has followed the life-histories of representatives (hatched from *Macrosiphum urticae*, Kalt., through *Aphidius ervi*, Hal.) of all the families listed above, including *Asaphes vulgaris*, *Coruna clavata*, *Lygocerus testaceimanus*, and *Charips victrix* in addition to others.

*Calculated from only 2 parasitised ; 14 healthy.

The Chalcids and Proctotrupids are ectoparasites of the Aphidiine larvae and pupae, attacking their hosts only after the Aphids are dead and the primary parasites have spun their cocoons.

The Cynipids, however, are endoparasitic and attack their hosts at an early stage and never when the cocoons have been spun.

Asaphes vulgaris, Walker.

This is an extremely common and polyphagous species. Walker (1835) described the adult (fig. 2).

Fig. 2.—*Asaphes vulgaris*, Walker. Size 1·9 × 3·3 mm. (ex *Myzus persicae*).

This species had been hatched from representatives of nearly all genera of the Aphidiines, more especially owing to their abundance, from *Aphidius* spp. The hosts are chosen after they have spun their cocoons and cemented the Aphids down, and eggs are laid singly on the upper surface of the larvae, or, more often, the pupae. Haviland states that each individual may deposit from 30 to 40 eggs which hatch in about 60 hours, the host dying a day or two after the larva begins to feed. Pupation lasts 14–16 days. This author claims that at least two generations a year may occur, depending upon the number of available hosts.

During the present survey, *Asaphes vulgaris* hatched up to 28 days after the *Aphidius* cocoons were collected but the greatest number appeared after 20 days. It first emerged from potato Aphids on 1st August (1945), and two generations can possibly occur on the potato alone ; all were hatched before the end of September. Adult females were found on many species of plants from the beginning of May to the end of October ovipositing into Aphids. The latter date suggests that the winter is passed in immature stages within the cocoons of the Braconid hosts.

The remains of *Aphidius* sp. could always be found in the cocoons from which *A. vulgaris* had emerged and in some cases it was clear that the death of the host had not occurred until the chitinisation of its pupa was fairly advanced, and always at a much more advanced stage than in the victims of *Lygocerus* spp. This indicates either a preference for later oviposition by *A. vulgaris* or the lapse of a longer period of time between oviposition and the death of the host than is the case with *Lygocerus*, more probably the former.

The size of the adult parasite (as mentioned under *Aphidius avenae*) varies with the size of the host, and evidence showed that the size of *A. vulgaris* may also be dependent on the stage in metamorphosis of the host when selected for oviposition. The size of an *A. vulgaris* female hatched from *Myzus persicae* is $2 \times 3 \cdot 9$ mm., and one from *Macrosiphum solanifolii* $2 \cdot 3 \times 4 \cdot 4$ mm., yet one from *M. rosae* measured only $1 \cdot 4 \times 2 \cdot 6$ mm. The last specimen had matured upon only part of the abdomen of a fully chitinised *Aphidius ervi* pupa which was obviously within a day of hatching before it was killed, leaving the thorax, head and appendages intact.

As in other Pteromalids, *Asaphes vulgaris*, was observed to feed upon its victim prior to oviposition.

Coruna clavata, Walker.

Walker (1833 and 1840) described and illustrated the adult.

This species was obtained only from the plot, through *Aphidius* sp. (probably *matricariae*), from *Myzus persicae*. It was also hatched from *Macrosiphum rubiellum*, Theo., and *Macrosiphum pisi*, Kalt., in both cases through the same primary host, *Aphidius avenae*, Hal. The life-history is very similar to that of *Asaphes vulgaris*.

Lygocerus testaceimanus, Kieffer.

The adult (fig. 3) is found from May to October. It is described by Kieffer (1914) and seems to be one of the commonest British species of *Lygocerus*.

Fig. 3.—*Lygocerus testaceimanus*, Kieffer. Size $1 \cdot 9 \times 3 \cdot 2$ mm. (ex *Myzus persicae*).

This species is an ectoparasite, generally upon Aphidiine larvae and pupae, but Haviland has found *L. cameroni*, Kieff., capable, due no doubt to faulty oviposition, of living upon pupae of its own species as well as those of *Asaphes vulgaris*, and this habit undoubtedly applies to *L. testaceimanus* as well.

Once more, only those primary parasites that have formed their cocoons are chosen as hosts, though seemingly at an earlier stage than with *Asaphes*, as inedible chitinised host remains are rarely found. The time taken for its life history, and the number of generations per year, are similar to those of *Asaphes*, but during the survey it was by no means as common.

The sex ratio 1 : 2 showed males to be in the majority.

All specimens of *L. testaceimanus* were hatched before the end of September, and winter may be passed immaturely in different host cocoons, or possibly some may survive as adults. Kieffer states that *Lygocerus aphidivorus* adults have been found overwintering under moss.

Lygocerus spp.

A few individuals of 2 distinct species with infumated wings were obtained.

Species A.

This was hatched from field counts on two occasions and both sexes were represented. Neither sex fits with satisfaction any of Kieffer's descriptions of species with infumated wings, though the female seems to be quite close to *fuscipennis*, Kieff., of which only this sex is described.

Species B.

This came from the plot only, hatching out of *Myzus persicae* through *Aphidius* sp. (*matricariae* ?). One specimen of each sex was obtained. The male is close to *frenalis*, Kieff., and the female to *flavipes*, Kieff., but Kieffer describes only these respective sexes of each species.

Charipids.

The species of this family listed above as being hatched from potato Aphids have the same life history. All are endoparasitic, and the eggs are laid in the larvae of *Aphidius* whilst these are in their first to fourth instars and their Aphid hosts are still alive. The presence of living Aphids, parasitised or not, causes excitement in ovipositing females, which climb on to their backs and test each with a cursory ovipositor thrust ; where the Aphid contains an *Aphidius* larva, oviposition takes place. Haviland states that only one egg is laid, and when more occur they are the result of other attacks. The present writer has dissected as many as six first-instar Charipid larvae from one mature *A. avenae* larva, though only one adult ever emerges. The growth of the *Aphidius* host is arrested immediately it has spun its cocoon and fastened the Aphid down. The Charipid when nearly full grown emerges from the host and after consuming the remainder of it pupates within the cocoon. The whole life cycle, during summer, takes about 30 days.

Winter is passed in a mature larval stage inside the cocoon of the host and the adult emerges in April or May of the following year.

Alloxysta sp.

This was hatched from the plot only ; it was not important.

Charips curvicornis, Cameron.

This species was found at one field centre where five specimens out of a total of 80 parasites and hyperparasites were hatched from *Myzus persicae*.

Charips tscheki, Giraud.

This was the only Charipid of importance in the garden plot, but it was never found in field counts.

Charips victrix var. infuscatus, Kieffer.

The adult (fig. 4) is very like *tscheki* but the radial cell of the fore wing is obviously longer. Dalla Torre and Kieffer (1910) give descriptions of both this and the foregoing species.

This was the most important species under field conditions but even so its numbers were never great.

The Importance of Hyperparasites.

The relationship of parasites and hyperparasites is very complex, and Haviland illustrates diagrammatically the state of affairs found when the primary *Aphidius* parasite is attacked by one or more secondary parasites.

Fig. 4.—*Charips victrix* var. *infuscatus*, Kieffer.   Size 1·3 × 3·1 mm. (ex *Myzus persicae*).

The endoparasitic Cynipids attack *Aphidius* larvae before they are full-grown, and therefore when present, fall victims, indirectly, to the attacks upon fully mature *Aphidius* larvae by the ectoparasitic Proctotrupids and Chalcids.   Cases occur where one *Aphidius* cocoon may be oviposited in by more than one ectoparasite of the same or different species.   Death generally ensues for all, if the stages of development of the ectoparasitic larvae are about equal, though sometimes the larger (or largest) kills the other (or others) and successfully reaches maturity.   *Lygocerus* is also capable of parasitising both the pupae of its own species and those of the Chalcids.

All or any of the above combinations may occur, but a straight attack on the primary parasite by only one ectoparasite is usual, and this primary parasite may or may not be the host of an endoparasite.

Anything attacking the primary parasites must be regarded as harmful, thus it is with ecto- and endo-secondary parasites should they occur by themselves.   However, where endoparasites are numerous, the ectoparasites by attacking hosts already parasitised by the former are beneficial, in that they kill these endoparasites (which have determined the fate of the primary parasites in any case).

Where potato Aphids were concerned, field counts showed that *Charips* were so much in the minority, that *Asaphes vulgaris* and *Lygocerus* spp. would in practice be killing only a negligible proportion of these endoparasites in their attacks on *Aphidius*, so all secondary parasites hatched can be grouped as harmful.

During the 1944 and 1945 survey, attacks were first noted towards the end of July upon the first *Aphidius* generation, and the ratio of the total percentage of primary parasites to hyperparasites hatched from all three species of Aphids was 44·6 per cent. : 55·4 per cent.   The hyperparasites were made up of *Asaphes vulgaris*

70 per cent., *Lygocerus testaceimanus* 20 per cent., and the remaining 10 per cent. by *Lygocerus* sp. A. and the two species of *Charips*.   The proportion of hyperparasites in 1945 showed a 12 per cent. increase over 1944.

Total hyperparasitisation was never encountered, and with only two host generations a year on potatoes it is very improbable that this would ever take place but it reduced the possible numbers of the second *Aphidius* generation, and supposedly the number of Aphids which might have been parasitised, by nearly half.

The Predators.

The only predators of account influencing Aphid populations in potato fields were Syrphids and Coccinellids, and only the former were found universally.

A. Syrphidae.

These very attractive and showy Diptera receive the first attentions of many collectors.   The family has been excellently monographed by Verrall (1901) but, in spite of their popularity and abundance, surprisingly little is known of the life histories of many species.   Bhatia (1939), however, dealt with several aphidophagous types, giving full descriptions of their bionomics and morphology, together with detailed drawings.

The species reared from eggs (or later stages) taken on the leaves examined during the field survey counts, were :—

Platychirus manicatus, Mg., *P. scutatus*, Mg., and *P. immarginatus*, Zett.

(*Melanostoma mellinum*, L., from the garden plot only.)

Sphaerophoria sp.

Syrphus balteatus, Deg., and *S. vitripennis*, Mg.

The white, elongated eggs are laid usually singly on the undersides of the potato leaves, and they hatch into slug-like larvae of characteristic form, which feed indiscriminately on any Aphid contacted whilst moving over the foliage.   They pass through three instars, and form their puparia on the leaves, young tips, or stems, of the potato plant ;   or having fallen to the ground, pupate amongst the surface material.   The adults are non-aphidophagous, feeding at flowers.

Other Syrphids (adults) observed in potato fields, whose larvae are known to prey on Aphids, were *Syrphus ribesii*, L., *S. corollae*, F., and *S. luniger*, Mg., and *Sphaerophoria scripta*, L.

Platychirus spp.

Bhatia (1939) claimed to be the first to record the genus *Platychirus* as aphidophagous, but an almost contemporary record of *P. peltatus*, Mg., found preying on *Cinara* (*Lachnus*) *cembrae*, Seitner came from Austria.   An important paper, also, by Fluke (1929) included, amongst many other Syrphid predators found attacking Pea Aphis in North America, three species of *Platychirus*.   It appears now, that all species of this genus will be found to be aphidophagous and many of the larvae prove difficult to differentiate on a colour basis alone.

Platychirus scutatus, Meigen.

The adult is common from the end of March until October in gardens and at flowers generally, especially in September.

Bhatia (*loc. cit.*) described the egg, larva and puparium and gave drawings of the two latter.   The third-instar larva is green, the dorsum with paler side lines and forwardly directed pinkish white V, or diamond, central markings.   The puparium is dirty brown, varying in size between $5 \cdot 4 \times 2$ mm. and $6 \times 2 \cdot 5$ mm.

The first egg was found in field counts on 18th July, and others occurred subsequently with a regularity only exceeded by those of *P. manicatus*. The more mature eggs hatched very soon after being taken, but many took 3 or 4 days. Approximately $2\frac{1}{2}$ weeks elapse before the larva is fully grown, when it stops eating, contracts slightly, remains in a torpid state for a week or more, and finally pupates. The adult emerges 2 weeks later.

Winter is passed in a suspended larval state (often, but not invariably, full-grown) and this suspension is at times begun in early September, despite ample food and high temperature. There is unlikely to be more than one generation on potato per season, and $1\frac{1}{2}$ appear to be the maximum. With no fixed time for the appearance of any stage, considerable over-lapping occurs, but a favourable year, it is thought, might produce an overall total of 2–3 successive generations, by the use of alternative Aphid hosts.

The exact number of Aphids consumed by one larva was not recorded, but this, it is expected, will fall between the numbers given for the two following species.

Platychirus manicatus, Meigen.

The adult is equally common everywhere in company with *P. scutatus*. Its life history has hitherto been unknown.

The eggs measure $1\times0\cdot4$ mm., and have the same lozenge-shaped elevations that Bhatia describes for *P. scutatus*. They are laid singly, normally on the under surface of the potato leaf, but are also found on the upper surface. As with *scutatus*, often 3 or 4 days are passed, after capture, before hatching, and an average of 15 days before maturity is reached. When full-grown, the third-instar larva measures $9\cdot3\times2\cdot4$ mm. It closely resembles the third-instar larva of *scutatus* in form, but the oblique, anteriorly pointing, dorsal stripes (on a whitish orange background instead of green) which carried the resemblance still further, have become diffused, and the larva assumes a fairly uniform straw colour, only the lighter lateral lines remaining. Like *scutatus* also, the first thoracic segment has 6 transverse dorsal hairs, the second none, and the third 8. The dorsal surface of the first 7 abdominal segments, however, each have a transverse row of 8 hairs (the middle pair placed slightly forward) and 2 lateral hairs (or sensory papillae ?) on either side (*scutatus* has 10 dorsal and 1 sensory papilla to each side).

The full-grown larva passes about seven days in a quiescent state before forming the puparium. The puparium is a lighter, cleaner, brown than that of *scutatus*, and broader. It measures $6\cdot5\times2\cdot8$ mm. The adult hatches in 14 to 16 days. One mature larva became somnolent on 3rd August, and overwintered thus. The winter seems to be passed in this state, and a search of various Aphid-infested plants in September and October revealed only very sluggish larvae at various stages of development, and no puparia. The number of generations per year must closely follow those given for *scutatus*.

At field centres, the eggs of *P. manicatus* were met with more frequently than those of any other Syrphid, and the larvae were found preying on a wide range of other Aphid hosts showing that the immature forms are quite as ubiquitous as the adults.

The diet of one larva was worked out in terms of *Macrosiphum pisi*, a slightly larger Aphid than *M. solanifolii*. The egg taken on 12th July hatched the same day and from then, until maturity, the larva was supplied with a carefully checked number of *M. pisi* each day. This supply included apterous and alate viviparous females, and their respective young stages, of which the instars were noted. The diet was :—

Apterous—13 mature, 4 (4th instar), 9 (3rd instar), 22 (2nd instar), 18 (1st instar). }71 total.

Alate—3 mature, 2 (4th instar).

Feeding is not continuous and only a few hours each day was so occupied.

The larva was fully grown by 23rd July, when it refused to eat any more and remained at rest, occasionally wandering round the tube, until it pupated on 1st August. It hatched on 15th August into a normal sized female.

Platychirus immarginatus, Zetterstedt.

The adult of this species is not common in the north. It was only encountered once during the survey and its life history, like that of *manicatus*, has to date been unknown. On this occasion an egg, which hatched in transit, was found on a potato leaf examined during the counting of a field in which the eggs of the other *Platychirus* spp. were present also.

The larva was reared on a recorded diet of *Macrosiphum pisi*, under the impression that it would serve as a duplicate feeding experiment for *P. manicatus*, which through-out its larval stages it showed every sign of being. It was, therefore, a surprise when the supposed *manicatus*, which appeared to be half grown, declined to eat, and later pupated, eventually hatching into an adult of *P. immarginatus*.

The larva hatched on 1st August, and fed until the 13th, rested until 21st August, when pupation took place ; the adult hatched 15 days later.

It consumed a total of 51 *Macrosiphum pisi*, made up of 5 mature, 7 (4th instar), 28 (3rd instar) and 11 (2nd instar) apterous viviparous forms.

As indicated above, the larva was so exceedingly like that of *manicatus* that no difference was noticed other than the much smaller size when mature. The puparium, also, was just a smaller replica of that of *manicatus*, being of the same shape and light brown colour and measuring $5 \times 2 \cdot 3$ mm.

Melanostoma mellinum, L.

The adult of *M. mellinum* is very like *Platychirus* but is distinguished by the abdominal markings of the female and the undilated fore-tibiae, or tarsi, of the male. It accompanies the two previously mentioned species of *Platychirus* and occurs in equal numbers. Voukassovitch (1925) recorded this species as predacious on the Cabbage Aphis, *Brevicoryne brassicae*, stating that it pupated on the leaves. He also supplies a list of its parasites.

As previously mentioned, *M. mellinum* was found only in the garden plot. Here all Syrphid larvae were noted under their family name, a more precise check being impracticable, so only its puparium is known with certainty. The puparium is elongate, with the posterior, basal, half often irregularly protracted. The average measurements are $6 \cdot 2 \times 2 \cdot 1$ mm. The colour is an apple-green, with most of the broader fore-end shining. When parasitised the colour turns a biscuit-brown.

Sphaerophoria spp.

There are three British species of this genus but not one of them is common in the Northern Province. The adult of *S. menthastri* var. *taeniata*, Mg., was hatched from the garden plot.

Only one larva was taken in the field and this proved to be parasitised so its specific identity could not be affirmed. This larva was the uniform smooth green characteristic of all three species, with a mid-dorsal pale lemon stripe widening half-way down the abdomen into a broader patch. At the centre where the larva was found, adult *S. scripta*, L., were seen. The puparium, however, was smaller than that given by Bhatia for *scripta*, being $5 \cdot 5 \times 2$ mm.

An unidentified Ichneumonid parasite hatched 30 days after its host pupated.

Syrphus spp.

The aphidophagous habits of this genus have long been known, and it contains species noted in all popular lists of "Gardener's Friends". The translucent white larvae of the common forms, with their specifically coloured and shaped dorsal patches are familiar amongst Aphid colonies infesting rose bushes. They make much more voracious demands for food than *Platychirus* spp. and eat continuously when food is abundant, maturing at nearly twice the rate of the latter. Their larger size at maturity also makes the total consumed greater.

Syrphus balteatus, Degeer.

The adult is extremely common everywhere from May to October, its unusual abdominal banding rendering it unmistakable. Bhatia deals with the larva and puparium ; the former is white with a slate-green dorsal patch, and the latter drop-like in shape, whitish with two or three orange dorsal markings.

The occurrence of immature forms in potato fields was more erratic than that of *P. manicatus*. But in certain fields all stages were found in numbers, and at these particular centres almost all the control exercised by predators could be attributed to *S. balteatus*.

Eggs took up to 3 days to hatch after collection. The larvae on hatching commenced to feed voraciously, and were fully grown in 6 days. Pupation began 1 day after feeding ceased and the imagines appeared after a further 8 days.

Thus, when Aphids abound, the complete life-cycle takes under 3 weeks, half that of *Platychirus* spp., and twice the number of generations given for the latter seem possible, though about 3 per year are more probable.

The winter is normally passed in the pupal stage, but it can also be passed in various larval stages.

An interesting observation was made in the garden plot on the effect of the amount of food available upon the rate of larval development. An approximately two-third's grown *balteatus* larva (the only one present at the time) was noted on 27th July, when the Aphid population had declined and little food was present. This larva took a further 14 days to reach maturity and pupate. Consequently the time taken from egg to pupa in potato fields where the Aphid population is low must be several times longer than the probable minimum time of 6 days under laboratory conditions with a glut of food.

Syrphus vitripennis, Meigen.

The adult is common in gardens from April to October. It closely resembles *S. ribesii*, L., an even commoner and more widely found species, and favours the same habitats.

The eggs were never taken, but one newly hatched larva and several older ones were found during August ; it was not, however, as common as *balteatus*.

In the absence of specimens of *ribesii* larvae no actual comparisons could be made but, in appearance, the third-instar larva of *vitripennis* follows closely the description of *ribesii* given by Bhatia. It has an orange, dorsal, wedge-shaped patch of variable size upon a whitish ground colour. In size, it is smaller on the average than *ribesii* and when fully developed measures $11 \times 2 \cdot 8$ mm. No difference could be found in the number of transverse hairs to each segment, being 6, none, and 8 on the thoracic segments, respectively, and 12 on each of the first 7 abdominal.

The puparium varies from light brown to brown, with occasionally orange V, dorsal markings. It measures $7 \times 3 \cdot 3$ mm.

Only one freshly hatched larva was reared ; it took 12 days to reach maturity, pupated after one day's rest, and the adult hatched 11 days later. During its larval life it was fed on a mixture of potato Aphids and consumed the following :—

Myzus persicae

>   Apt.—23 mature ; 5 (4th instar) ; 2 (3rd instar).
>   Alate.—2 mature.

Macrosiphum solanifolii

>   Apt.—37 mature ; 14 (4th instar) ; 6 (3rd instar) ; 1 (2nd instar).
>   Alate.—12 mature ; 4 (4th instar).

Aulacorthum solani

>   Apt.—2 mature ; 2 (3rd instar) ; 1 (2nd instar).
>   Alate.—1 mature.

Total.—112.

In common with *balteatus* any living material contacted seems to be tested for edibility. Bhatia states that only in cases of extreme hunger is cannabalism resorted to amongst Syrphid larvae but the present author on one occasion found that two *vitripennis* larvae tubed in the field with a plentiful supply of acceptable Aphids, had, an hour or so later, nearly consumed a smaller larva of their own species which was in the same tube. On another occasion when a well-developed larva of *vitripennis* happened to be in the same tube with an adult of its own kind, the former became attached to the joint between the coxa and trochanter of the fly's hind-leg, and clinging tenaciously, rendered the leg useless before the victim freed itself.

Winter is probably passed as in the case of *balteatus*.

The Occurrence of Syrphids.

The normal number of eggs found on 100 leaves examined in each field was 2 or 3. In one field the number was 9, and several complete plants examined here at random showed an average of 3 eggs per plant.

Platychirus manicatus and *P. scutatus* were constant control factors, the former being the more common but neither in themselves exerting much check. *Syrphus balteatus* was more infrequently met with but, when it did appear, often did so in numbers, and then its control value was the greatest of all the species. The other Syrphids mentioned were complementary, with *S. vitripennis* the most important.

Syrphid eggs were found from mid-July to the end of August, thus, like the parasites, the increase in Aphid populations had proceeded unhindered by the attacks of these predators during the critical initial period of a month or more ; when they become numerous, Aphid infestation is well advanced and, at times, declining from a maximum.

Parasites.

None of the eggs taken was parasitised, and the only larva found parasitised was one of *Sphaerophoria* sp. As it was chiefly the eggs which were collected, lack of information as to the percentage larval-parasitism is natural ; a few observations made on Syrphid parasites in the garden plot will be dealt with separately.

B. Coccinellidae.

In this well-known " ladybird " family both the adults and larvae of most of its members are predacious. Those found on potato Aphids were *Coccinella septempunctata*, L., and *Adalia bipunctata*, L., the latter only in the plot, and then rarely.

Coccinella septempunctata, L.

The adult of this species is the commonest of all British ladybirds as well as the most cosmopolitan. It appears from May to October amongst Aphid colonies on many plants. Its yellow eggs are laid in clusters on the undersides of leaves, and the larvae take about 4 weeks to mature, feeding on Aphids the while. They vary in colour from light grey to nearly black, most often the latter, with an orange patch on each side of both the first and the fourth abdominal segments. Pupation takes place on the leaf or stem and the pupae also vary greatly in colour, from yellow-orange with black markings to almost all black. The adults hatch from 7 to 12 days later. One complete life cycle is all that seems to take place on the potato, the adults appearing from mid-July, and their progeny (as adults) a month later. Winter is probably passed by the adults in sheltered crannies, amongst leaves in hedge-bottoms, etc., as with many beetles.

Swarms of *C. septempunctata* are often found where field crops are Aphid infested, and where they appear, the adult beetles and their larvae generally eradicate the whole infestation. During 1945, these swarms were particularly common. The first was noted in late July on barley heavily infested with *Macrosiphum granarium*. Very soon afterwards, another swarm was seen busily feeding on *Myzus persicae*, which thickly coated the undersides of most leaves in a late-sown turnip crop. From then until mid-August extensive numbers of both pupae and fully grown larvae were reported from many potato fields chiefly by the owners alarmed at the profusion of these "unknown creatures" and suspecting Colorado beetle. In these fields, the potato Aphids, which must obviously have induced this plethora of Coccinellids, had either been entirely wiped out, or soon would be. In the centres where survey counts were made, however, *septempunctata* was no more than a contributory controlling factor and was found late in the season in only the minority of fields. It was never found in 1944 at the centres visited, but Jacob (1944) records that it occurred in very great numbers in the Tees area and resorted to cannabalism after eliminating the Aphids.

Larvae reared on *Macrosiphum solanifolii* consumed an average of 100 Aphids, made up of about 16 (2nd instar), 13 (3rd instar), 28 (4th instar) and 43 adult, apterous forms, approximating to the amount given for *Syrphus vitripennis*. No feeding records of adult *septempunctata* were obtained.

Phalacrotophora fasciata, Fall., a Phorid parasite of Coccinellid pupae, is recorded in certain years as being prevalent but none of the dozens of pupae collected in 1945 proved so parasitised.

THE GARDEN PLOT.

Up to now the subject has been treated as far as possible against the wider background of field conditions, as it was felt unwise to mix the necessarily more generalised field observations with the more detailed data obtained from the plot. The increased number of host-plants for both potato and other Aphids, found in the garden, resulted in a wider range of aphidophagous insects and produced artificial conditions not met with in the field. This provided an interesting study in itself. Many of the results, however, have common application and serve to crystallise some of those obtained from the field.

The plot consisted of 3 short rows, 9 potato plants per row, running in an east to west direction and set at normal planting distance both in and between the rows. A wall, rhododendron bushes and a wide path made up three sides, and all at about one yard distance ; bare ground formed the fourth side. The danger of infestation from crawling apterous Aphids, could therefore be discounted.

Tubers of the variety Bintje were sprouted and completely Aphid-free when planted on 15th April. The first shoots broke the surface on 9th May, and the first examination was made ten days later. Complete inspections followed weekly until 3rd August,

and less systematic ones after this date. Separate Aphids counts were kept for each plant, though these have been grouped together as a whole, for there is certainly much interchange of insect denizens when potato-haulms touch and begin to mingle.

The numbers of alates, apterae and young were also kept, but for brevity these are totalled in Table I. Figures for 12 complete counts are given in terms of healthy,
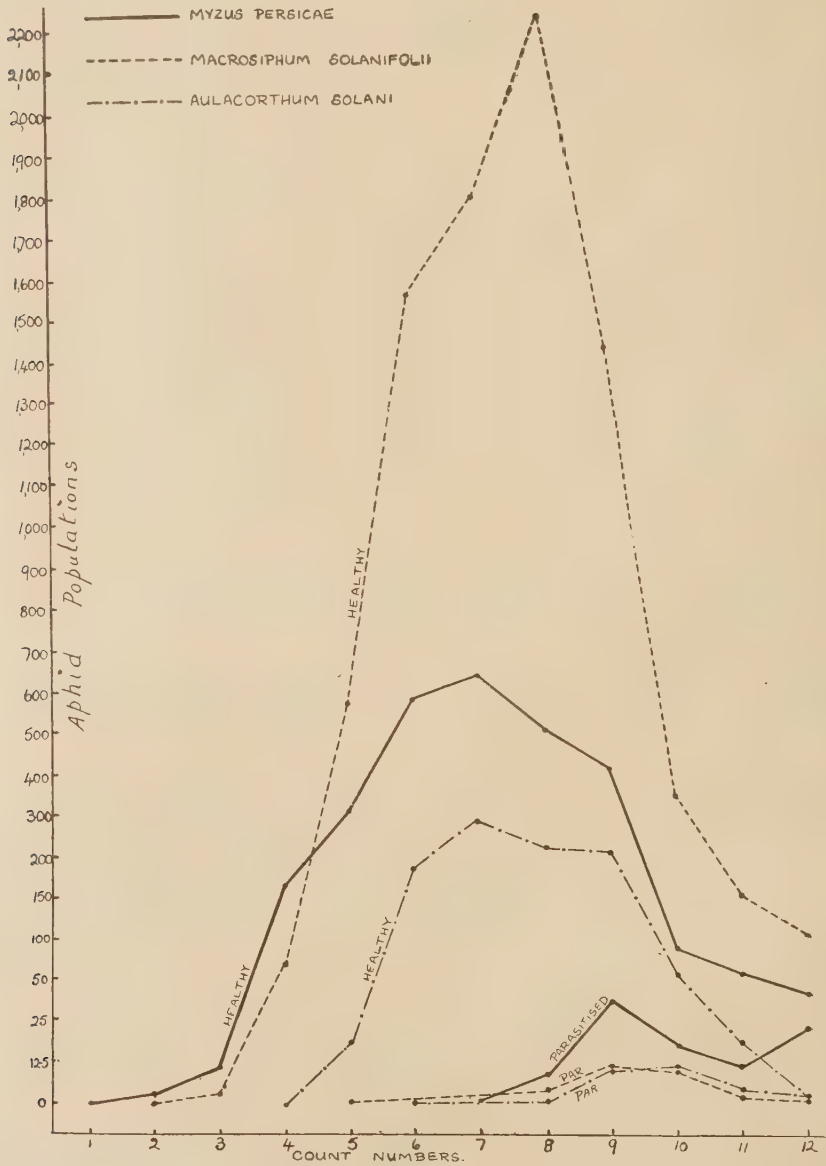
Fig. 5.—Graph showing populations in garden plot.

TABLE I.

Count and Date	Myzus persicae H.	P.	F.	Macrosiphum solanifolii H.	P.	F.	Aulacorthum solani H.	P.	F.	Predators Syrphid E.	L.	Pu.	Others
1. May 19 …	—	—	—	—	—	—	—	—	—	—	—	—	—
2. May 25 …	3	—	—	—	—	—	—	—	—	6	—	—	—
3. June 1 …	11	—	—	3	—	—	—	—	—	5	—	—	—
4. June 8 …	169	—	—	72	—	—	—	—	—	42	—	—	—
5. June 15 …	313	—	—	574	1	—	16	—	—	38	14	—	—
6. June 22 …	588	—	—	1,568	—	1	185	1	—	47	17	—	—
7. June 29 …	639	2	—	1,798	—	1	292	—	—	36	18	—	—
8. July 6 …	512	9	—	2,230	5	7	225	2	—	12	40	1	1 Chrysopa carnea cocoon. 1 Anthocoris sp. nymph. 1 Adalia bipunctata larva.
9. July 13 …	417	37	2	1,436	12	42	210	·11	1	30	18	9	1 Adalia bipunctata pupa. 2 Staphylinid larvae.
10. July 20 …	90	16	—	351	10	14	60	12	1	18	1	3	2 Adalia bipunctata larvae and 1 adult. 10 Staphylinid larvae.
11. July 27 …	62	12	1	154	3	2	18	5	1	16	3	1	1 Adalia bipunctata larva.
12. Aug. 3 …	43	24	1	107	3	3	4	4	—	16	1	—	—
Total …	2,847	100	4	8,291	34	70	1,010	35	3	266	112	14	—
	96·49	3·37	0·14	98·76	0·41	0·83	96·37	3·34	0·28	Per cent. of each Aphid, H : P : F.			

H = Healthy; P = Parasitised; F = Fungus attacked; E = Eggs; L = Larvae; Pu = Pupae.

parasitised and fungus-attacked specimens of all three Aphids. The eggs, larvae and pupae of Syrphids, and other predators are also noted, against the date when each count was started (always on a Friday evening and continued over the week-end). The graph is adapted from the numbers of healthy and parasitised Aphids given in the table.

A. APHIDS.

It will be seen that, although it arrived first, *Myzus persicae* increased more gradually than the other two species. *Macrosiphum solanifolii* appeared one week later than *persicae* and reached its peak correspondingly later. *Aulacorthum solani* was the last to arrive but its greatest numbers coincided with those of *persicae*. Although no figures have been given in the table, the greatest influx of alates of all three was on 15th June. Those of *persicae* reached 150, the highest total, but fell from this maximum more rapidly than those of *solanifolii* and *solani*, which were 134 and 8 respectively. When the numbers of these alates, which cause the initial infestation, are viewed against the maximum populations reached during the summer, *solani*, though never so numerous, appears the most efficient coloniser, even more so than *solanifolii*, and *persicae* distinctly the least.

By 3rd August, the potato haulms were becoming tangled and straggling over the ground ; some were withering due to slug attacks at their bases and the number of Aphids had declined to almost a minimum. Additional observations showed that all three species persisted sparingly and died out with the foliage.

Vagrant alates of *Drepanosiphum platanoides*, Schr., *Macrosiphum rubiellum*, Theo., *Macrosiphum rosae*, L., *Eriosoma* sp., *Pemphigus* sp., and *Cryptomyzus ribis*, Oest., were all encountered but either these died of starvation or flew off.

A few alate *Doralis fabae*, Scop., were found from 15th June onwards ; these produced a limited number of young, which also reproduced subsequently, but they never really managed to establish themselves. *D. fabae* also occurs at times in field counts. No specific work has been carried out on its potentialities as a transmitter of potato virus diseases ; it is a vector of certain other plant viruses however.

Myzus ornatus, Laing, appeared and began replacing *M. persicae* about mid-July remaining until the end. This species did not reproduce quickly and favoured the older leaves where it kept closely to the leaf-veins. Its smaller size, more rugose dorsum, and shorter, sinuate cornicles help to distinguish it from *persicae* which it resembles very closely. It appeared erratically in small numbers at field centres. Records show that it can convey potato virus Y and leaf-roll.

Alates of *Brachycaudus helichrysi*, v. d. Goot, were found in all of the first 9 counts. Some of these produced a few larvae which developed on potato.

B. FACTORS INFLUENCING THE INCREASE OF THE APHIDS.

(i) **Parasites.**

Parasitisation was not high, and the table shows that the largest number of parasite cocoons corresponded to the maximum numbers of Aphid hosts found about a fortnight before, though, of course, the proportion of cocoons to healthy Aphids increased as the Aphid populations began declining.

The three species of Aphids yielded respectively :—

Macrosiphum solanifolii

    Out of 34 parasitised   ...   24 *Aphidius avenae*, 7 *A. ervi* and 2 *Asaphes vulgaris*.

Aulacorthum solani

>   Out of 35 parasitised   ...   20 *Aphidius matricariae*, 6 *A. avenae*, 2 *A. ervi*, 1 *Praon volucre* and 5 *Charips tscheki*.

M. persicae

>   Out of 100 parasitised   ...   57 *Aphidius matricariae*, 1 *A. avenae*, 1 *Praon volucre*, 3 *Asaphes vulgaris*, 2 *Coruna clavata*, 2 *Lygocerus* sp. B., 22 *Charips tscheki* and 2 *Alloxysta* sp.

(The remaining cocoons of all three failed to hatch.)

Aphidius matricariae, Hal., was most probably introduced into the plot by the alates of *Brachycaudus helichrysi*. On 22nd June, out of 20 alates of *B. helichrysi*, 11 were parasitised by *A. matricariae*, a remarkable proportion. These must have been oviposited into by *matricariae*, in their late nymphal stages, developed wings and migrated, transporting the young parasites with them, for they were apparently healthy on arrival and a day or two later became fixed to the potato leaves in the form of *Aphidius* cocoons. Their origin is unknown, but they might easily have migrated from greenhouse chrysanthemums upon which they had overwintered.

Aphidius avenae, Hal., showed a preference for *Macrosiphum solanifolii* but in the field this was not apparent. *Aphidius ervi*, Hal., attacked only *M. solanifolii* and *A. solani*. Only two cocoons of *Praon volucre*, Hal., were taken, but later examinations gave more.

The hyperparasitic Cynipids were hatched chiefly from *M. persicae*, and these most certainly through *Aphidius matricariae*, while those hatched from *Aulacorthum solani* can reasonably be supposed to have used the same host, not only because *matricariae* was hatched more frequently from this Aphid but also from the fact that the Cynipids were never hatched from *Macrosiphum solanifolii*, whose primary parasites were exclusively *Aphidius avenae* and *A. ervi*. It is uncertain whether size or species of host determined this preference. Hyperparasites appeared first in the 9th count, *i.e.*, when most *Aphidius* cocoons were found.

(ii) **Predators.**

Syrphid eggs were laid on plants before any Aphids appeared and a total of 6 eggs was found on 25th May, when the only Aphid on the potatoes was a newly arrived alate *M. persicae* which had produced two young. Eggs were found right through the season until the foliage died.

Figures in Table I show that, as with the parasites, the greatest numbers of larvae found corresponded to the greatest supplies of food. Counts 11 and 12 reveal a decrease of eggs and larvae but freshly laid eggs were again numerous on 10th and 17th August, apparently the result of the increasing numbers of adults hatching from summer broods.

The only species of *Syrphus* that developed on the potatoes was *S. balteatus*, Deg. A puparium was taken on three occasions, 13th, 20th July and 10th August.

Apart from a single puparium of *Sphaerophoria menthastri*, L., taken in September, all the other puparia were those of *Platychirus scutatus*, Mg., and *Melanostoma mellinum*, L. All the larvae seen, and presumably the vast majority of the eggs, were those of *Platychirus* and *Melanostoma*. The larvae of the two genera are undoubtedly similar, and with *Platychirus* at least, the species remain in a torpid condition for a week or more before pupating. In such a state, they do not adhere well to the leaves and are easily knocked off (a heavy shower is sufficient to do this), and falling to the ground pupate in the surface soil, or debris. If they do manage to remain they often pupate on the young unexpanded leaves at the shoot-tips.

Hence while only the puparia of *P. scutatus* and *M. mellinum* were collected from the leaves, this does not necessarily bear upon the number of species of these genera which were present. Larvae of *P. manicatus*, Mg., were certainly there, while of the three, *P. scutatus* and *M. mellinum* predominated.

The 14 puparia collected from counts 1–12 were : 8 *P. scutatus*, 4 *M. mellinum* and 2 *Syrphus balteatus*.

Two *P. scutatus* and two of the *M. mellinum* puparia were parasitised, one of each by the Ichneumonid, *Bassus laetatorius*, F. (the other parasites failed to hatch). The adult female of this well-known parasite of Syrphid larvae oviposits into its host while it is either an early larva, or in a late embryonic stage within the egg. The parasite larva follows through its host's larval life and pupates in the puparium of the latter, hatching 3 weeks after this is formed.

Parasitised host larvae develop up to pupation stage with their appetites for Aphids seemingly unimpaired, so it is also with suitably mature host eggs. But should the egg be too freshly laid, *B. laetatorius*, after making the usual two or three test insertions with her ovipositor, discovers its unsuitability, turns round and devours it, leaving only a crumpled, empty chorion. The number of life-cycles per year of this parasite will depend on those of its host ; the late summer generations of Syrphids often reveal a very high percentage of parasitisation. During autumn 1945, *B. laetatorius* was especially active among Syrphid larvae on many species of plants, occurring often in numbers large enough to produce total parasitisation, and one female methodically scouring Aphid-infested leaves for hosts, was seen to consume more Syrphid eggs than she oviposited into.

Adalia bipunctata, L.

Table I shows that this Two-Spot Ladybird occurred in July, but only in small numbers. It was present amongst Aphids on other plants in the garden from June to October, and has a life-history similar to that given for *Coccinella septempunctata*, though it was never found in field counts.

The larva is smaller and consistently darker than that of *C. septempunctata* with only one orange patch on the fourth abdominal segment, placed mid-dorsally ; the pupa is also smaller and more clearly marked.

Clausen (1916) carried out experiments on this species in California. He found that, on the average, the eggs hatched in five days whilst the four larval instars occupied a total of 17 days and the pupal stage 6. Also that the larvae, supplied with medium sized *Macrosiphum rosae* devoured a maximum of 308 and a minimum of 220 with an average of 252 ; the corresponding figures for the adult beetles being maximum 305, minimum 215 with an average of 251.

Chrysopa carnea, Stephens.

This is the common green " Lacewing " ; it is fully described by Killington (1937) in his monograph on British Neuroptera.

One cocoon was found on 6th July attached to the young leaves at the tip of a potato shoot. The larva (which had escaped detection on previous counts) could be seen rotating inside, thickening the inner wall with more silk. This white and completely globular cocoon was opened by a neatly cut lid on 19th July, and the mature pupa emerged, to cast its skin and change into an adult an hour later.

This is the only British lacewing known to hibernate as an adult. Killington suggests at least two broods per year, and it seems unlikely that any more occur in the north ; the whole life-cycle probably takes 2 months or more usually but it can be completed in six weeks. He also gives Withycombe's figures for the amount of Aphids required for full larval development as being 111 to 142 full-grown female *Aphis rumicis*, L. (*Doralis fabae*, Scop. ?). The adults are also predacious on Aphids.

Kimminsia subnebulosa, Stephens.

This Hemerobiid was the commonest lacewing in the garden during summer and the adult was found as late as December.   One or two of their yellowish green eggs, which are laid against leaf-veins and are not pedicellate as are those of *Chrysopa*, had been previously noted in other examinations but had evidently succumbed to attacks by other predators for the larvae could never be located.

Two larvae, one newly hatched and the other a little older, were found on a plant in the potato plot on 1st September.   These two larvae were tubed separately, and reared.   The newly hatched larvae took nine days to mature and in the process consumed two mature and two 2nd-instar *Myzus persicae* apterae, one alate, and five mature, two 4th-instar, one 3rd-instar and two 2nd-instar apterous *Macrosiphum solanifolii*, one apterous *Aulacorthum solani*, one Syrphid egg and one 2nd and one 3rd instar Typhlocybid nymph.   It spun a loose oval cocoon from which the Cynipid parasite, *Aegilips dalmani*, Reinh., hatched 48 days later.   The other larva proved to be similarly parasitised.   *A. dalmani* has not hitherto been recorded from this host, but the adults were common in the garden during June and again in autumn, amongst several Aphid colonies upon which *K. subnebulosa* was preying.   Neither of the larvae reared showed any signs of being parasitised and attacked the Aphids offered, quite normally.   The smaller one must have been oviposited into, either just after hatching or in the egg stage.   According to Clausen (1940), there is only one recorded instance in Cynipoidea of oviposition in the eggs of the host, that of *Ibalia leucospoides*, Hoch., parasitic on *Sirex cyaneus*, F.

In the Northern Province two generations of *K. subnebulosa* are probable and mature larvae were found hibernating under willow-bark, within their cocoons ; and part-grown ones, in the bark crevices of apple trees in late December.

Anthocorids (Hemiptera).

The control value of this family appears to be of little importance where potato Aphids are concerned.   The nymph of *Anthocoris* listed in Table I seemed about third instar and was found preying upon a very young *Macrosiphum solanifolii*. *A. nemoralis*, F., and *A. sylvestris*, L., were common in the garden throughout the summer and this nymph would most likely be of one of these species.

Cecidomyiids (Diptera).

This family likewise produced what must be termed an " also found " in the form of one larva during September.   It was an interesting and surprising find, because Cecidomyiids, owing to their poor locomotory powers, favour large Aphid colonies where food is to be had in plenty.   Many colonies of Aphids on various garden plants fell victims to the larval attacks, and had some appeared on the potatoes in June, they would have done excellent work, especially amongst *Macrosiphum solanifolii* colonies on the flower heads.

They attack their Aphid hosts normally at a leg-joint and kill very quickly.   In a glut of Aphids they appear to develop a lust for killing, and pass quickly from one Aphid to the next without stopping to feed.   A tightly clustered colony of *Doralis fabae*, Scop., extending for about 9 inches down a shoot of *Philadelphus coronarius*, was wiped out in this way by only three Cecidomyiid larvae ; each Aphid was left in perfect condition, hanging to the host-plant by its embedded rostrum.

The larva taken from the plot was found on the underside of a leaf in the middle part of the plant, busily sucking an apterous *M. persicae* adult.   Other Aphids were scattered sparingly over the whole plant but there were by no means any " colonies ". It seemed nearly full grown and was placed in a tube with an apterous female of each of the three major potato Aphids ; it killed them all, and partially sucked out the specimen of *Macrosiphum solanfolii* before pupating but unfortunately failed to hatch.

Staphylinids.

Larvae which Dr. van Emden of the Commonwealth Institute of Entomology kindly identified as *Tachyporus* sp. were found on counts 9 and 10, roaming the potato foliage generally. They looked like scavengers and have been included because one was discovered attacking a parasitised *Aulacorthum solani* ; having torn a hole in the dorsum of a cocoon, it had eaten the abdomen and some of the legs of the fully chitinised *Aphidius avenae* pupa inside, by the time it was disturbed. Later, though taking unkindly to captivity when placed in a tube, it was also found to eat Syrphid eggs.

Araneida.

During the counts several spiders were noticed to have spun webs from plant to plant, their number on 6th July was 31, and many webs contained alates of all three Aphid species. Webs spun early in the season probably constituted the first check upon possible Aphid populations by entrapping arriving alate progenitors.

(iii) **Entomogenous Fungus.**

This factor helps to complete the picture of biological control, and in the case of · *Macrosiphum solanifolii* was more important than parasitism, and would have been considerable had the 1945 summer provided suitable moist and warm weather for its spread. It was of slight account with the other two species (Table I).

There appear to be at least two types of fungi attacking potato Aphids. The type found in the plot which turns the victim a salmon pink colour and has little external mycelial growth. This it is thought, was introduced by an alate *Macrosiphum solanifolii*, which was the first form found to be attacked, and its subsequent hosts were chiefly of this species.

The other type was found to a limited extent in the field, and seemed to favour *Myzus persicae* more than the other two species. The host just after it has been killed looks fairly normal upon the potato leaf except that it is unusually immobile · and the antennae extend unnaturally forward. Later, a thick white mycelium completely envelops the Aphid, obscuring its identity.

(iv) **Rain.**

This is a natural factor which might at times curb Aphid populations, but during the first 12 counts its effect appeared negligible, though heavy rain did fall during that period.

Counts made on the first 10 plants in the plot on 15th July, before a heavy all-night downpour, showed no noticeable difference in number of Aphids when these same plants were counted the day following, and displayed a similar proportionate decrease over the previous week's count as did the remainder of the plot counted after the downpour. Several later sown potato plants elsewhere in the garden, however, which were heavily infested with Aphids in August, were almost completely cleared when lashed by wind and driving rain.

Summary.

The parasites and predators found attacking the Aphids, *Myzus persicae*, *Macrosiphum solanifolii* and *Aulacorthum solani*, during field survey work of potato Aphids in the Northern Agricultural Advisory Province during 1944 and 1945, are treated individually, receiving the proportionate attention which their occurrence merits.

Aphidius avenae was the most important primary parasite, and in potato seed growing areas, the only one ; while *Asaphes vulgaris* in particular, augmented by *Lygocerus testaceimanus* composed the majority of hyperparasites. The effect and relative importance of both types of parasite upon the Aphid populations of the survey are discussed with their percentage figures.

Predacious Syrphids included *Platychirus manicatus* and *P. immarginatus* whose life-histories were previously unknown ; feeding records are also given for these two, and the former species accompanied invariably by *P. scutatus* occurred regularly on potatoes. More unusually, but often more effectively, *Syrphus balteatus* appeared. Other species were found.

Coccinella septempunctata presents a total check when it occurs in swarms during early August, but predators and parasites appearing from about mid-July seldom interrupt the growth of Aphid populations until the infestation is stable or declining.

Potatoes grown in a small garden plot give a more detailed, if miniature, indication of the type of natural balance achieved by the indigenous species of insects. One or two results from this plot have overlapped into the main part of the paper, but the new factors (fungus and rain) and most predators and parasites found here, are dealt with separately as far as possible, and their individual importance denoted by a table of 12 complete counts. A graph supplies population curves for healthy and parasitised Aphids. Other Aphids found on this plot are mentioned, and the overall results emphasise again that the combined native enemies interfere with their Aphid hosts at too late a stage to prevent colonisation.

Acknowledgements.

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AN EXPERIMENT IN CONTROL OF TSETSE WITH DDT-TREATED OXEN.

By E. F. Whiteside, B.Sc.

Department of Tsetse Research, Tanganyika Territory.

The object of this experiment was to ascertain whether extermination of tsetse-flies (*Glossina*) could be brought about by introducing into their habitat large numbers of DDT-treated oxen.

It was arranged that, in the bush chosen as the site of the experiment, the introduced oxen should greatly outnumber the larger and more conspicuous game animals, in the hope that a majority of tsetse-flies would attempt to feed on oxen and die, and that the continuous toll so taken would lead to extermination. (Thus in principle this measure is similar to " hand catching ", *e.g.* Symes and Vane 1937.) Oxen competing with game for the fly's attentions were chosen for two reasons. Firstly, they are one of the most favoured domesticated hosts (Vanderplank 1944) and by virtue of their scent and movement might be expected to attract large numbers of tsetse ; secondly, by the use of Phenidum Compound (M & B S 897) they may be kept in tsetse-bush with considerable safety, at least under local conditions.

The use of oxen permits greater economy of equipment and materials than widespread applications of DDT to the bush and has a less widespread and incalculable effect on the general animal community. It is, of course, merely one method of restricting the use of insecticide to situations where large numbers of tsetse continuously resort. Vanderplank (1947) has discussed both this and other ways of using DDT against tsetse.

Somewhat similar experiments have been carried out in America, chiefly against the horn fly, *Siphona irritans*, L. Wells (1944) in Texas, Matthysse (1946) in Florida, and Laake (1946) in Kansas treated ranch cattle with aerosols, emulsions and suspensions of DDT by spraying or dipping on a field scale. In general these measures appear to have been successful ; 1 per cent. concentrations of DDT kept horn fly on cattle at insignificant levels for several weeks after application. The problem with tsetse, however, is different and more difficult. Extermination—not merely " control "—is required ; the contact of tsetse with cattle is relatively fleeting—perhaps only 1 minute in four or five days—compared with that of horn fly ; and whereas against the latter it is sufficient to treat the heads and backs of cattle, against tsetse the legs and belly are most important, and are difficult to spray efficiently.

Experimental Details.

Site of Experiment.

Block 4 (A and B) at Old Shinyanga, Tanganyika Territory, consists of 5 square miles of thorn bush already adequately described by numerous authors (Swynnerton, 1925, 1934, 1936 ; H. M. Lloyd, 1935 ; Vicars-Harris, 1936 ; Potts, 1940)—which is denser than normal because fire has been excluded for many years. It contains a flourishing population of *Glossina pallidipes*, Aust., against which the experiment was directed, and small numbers of *G. swynnertoni*, Aust. It is isolated from the nearest neighbouring tsetse bush, blocks 10B and 10C, by at least 1½ miles of clearing all round. Tests on a large scale simultaneously with the DDT experiment showed that a negligible number of *G. swynnertoni* managed to cross the clearings, and the experimental results themselves show that few or no *G. pallidipes* could have been crossing. The site of the experiment was therefore sufficiently isolated for reliance to be placed on the results.

DDT-treatment of oxen.

A solution containing 9 per cent. w/v pure DDT* and 9 per cent. w/v resin in commercial groundnut oil was used. The resin was of unknown origin and composition ; a sample has been sent to the London School of Hygiene and Tropical Medicine. The groundnut oil was heated to 105–110°C. while dissolving the DDT and resin, and the resultant solution applied (cold) to oxen at the average rate of 110 cc. per ox, excluding wastage, corresponding to $9\frac{1}{2}$–10 grams pure DDT per ox, or about 450 mg. per square foot of body surface. The head alone was left unsprayed.

This solution is not very efficient ; it quickly loses its lethal power (for short contacts with tsetse) and is theoretically wasteful of DDT. But since by repeated application it could render oxen continuously and highly lethal, the search for better preparations was left until the possibility of their successful employment against tsetse has been investigated.

Grazing and spraying organisation.

Altogether 340 oxen were used, 68 to the square mile or about 1 to 10 acres. This is estimated to be about 6 times the indigenous large-game population (mostly giraffe,

10 = *Herd of (10) oxen*
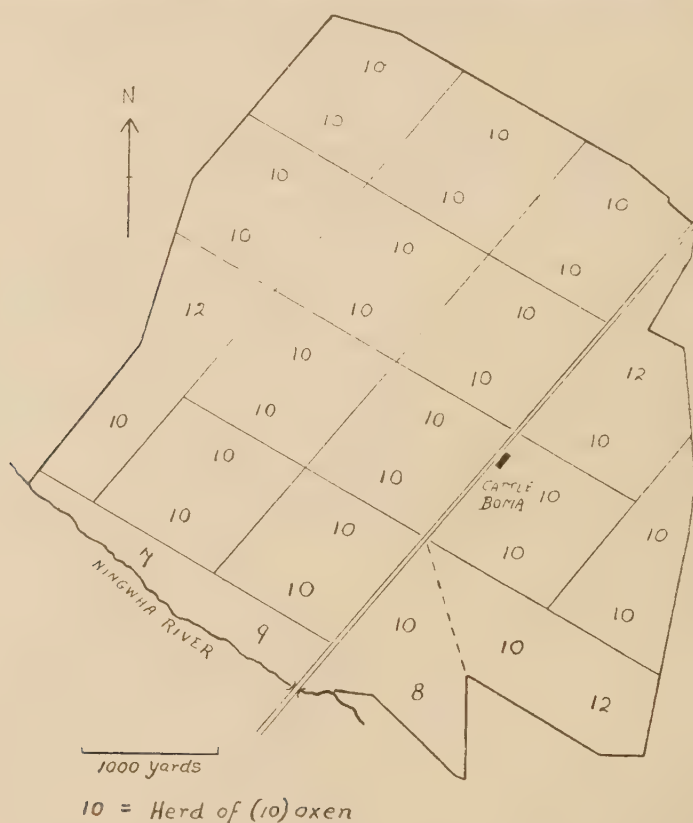
Fig. 1.—Plan of the experiment block (block 4, Old Shinyanga). The distribution of herds of treated oxen is shown as in Phase I ; two herds in each square grazed the northern and southern halves separately. During Phase II each of the herds shown was divided into two, each grazing a quarter of a square.

*2,2-bis (parachlorophenyl) 1,1,1-trichloroethane : amounts of commercial **DDT** were adjusted to give 9 per cent. of the para-para isomer in solutions.

greater kudu and impala) but less than the small game (pig, warthog and small buck).

By dividing the block into squares (see fig. 1) it was arranged that a herd of 10 oxen occupied each 1/6th square mile. Later, there were 5 oxen in each 1/12th square mile. (These elaborations were undertaken in order to give the measure every chance of success, and not necessarily with a view to their use in practical reclamation.) Oxen were in the bush for about 10 hours daily, from 7.30 a.m. to 5.30 p.m. Large numbers of herdsmen—the most expensive item—and a few headmen and game-scouts were employed, the latter for protection against carnivora (which gave no trouble). The oxen passed down a narrow railed passage during spraying at one end of which two Africans treated them singly, using sprayguns driven by compressed air from a small petrol engine. The over-all time needed was $1\frac{1}{2}$ minutes per ox. The cattle were kept out of the bush for half a day a week, when sprayed once weekly, and for one day a week when it was twice as often, in the second phase of the experiment.

Every week blood slides from oxen in poor condition were examined for trypanosomes. Oxen giving positive slides received a single dose of Phenidium 7 days later. No cattle were lost from trypanosomiasis, only 5 from accidents, and the majority visibly improved in condition in spite of living for 5 months in tsetse-infested bush. Evidence that the local cattle possess considerable resistance to trypanosomiasis appeared during the experiment, and this resistance probably played some part in preventing losses from the disease.

In passing, it may be noted that, in the second half of the experiment, oxen receiving nearly 20 grams of pure DDT on their skins weekly for 2 months appeared to suffer no ill effects.

Observation of the tsetse population.

Forty-eight random catches a week took place in block 4. A catch was confined to a single grazing square and was made by two fly-boys with a black cloth screen moving at random for two hours between 8.30 and 11.00 a.m. Sixteen of the grazing squares were each sampled 3 times weekly in this manner, so that incidence of destruction in different parts of the block might be followed. In block 10C, $1\frac{1}{2}$ miles distant, used as a control, 30 similar catches took place weekly. No danger is to be apprehended that these rates of catching would affect the population directly, since *G. pallidipes* comes little to man. The number of non-teneral* females caught was taken as an index of the population.

Course of the experiment.

Catching began 7 weeks before, and continued 4 weeks after, the experiment proper. Treated oxen were first introduced on 4th February, 1946 (during a poor rains) and remained continuously until 1st July. For the first 13 weeks (Phase I) they were sprayed once weekly and herded in tens. At the end of this period the results were reviewed and a second phase was begun on 6th May, continuing for 8 weeks. In this phase oxen were sprayed twice weekly, to raise their lethal power, and herded in fives, to raise the chances of contact with tsetse. Their lethal power was continuously checked by a technique previously developed. In Phase I about 70 per cent. of tsetse contacting oxen were probably killed, and in Phase II about 95 per cent.

Assessment of Results.

Since tsetse populations (as shown by catches) fluctuate quite widely over a period of months it is not easy to measure an artificially induced reduction accurately,

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*A teneral tsetse is one which has recognisably never had a meal.

for it may be masked or intensified by natural trends. Some accuracy was desirable, however, in order to estimate probable times required for extermination, since this was not in fact achieved. The method of assessing the results is therefore somewhat complicated.

Non-teneral females in this particular instance show most accurately what is accomplished, for ultimately they govern the numbers of non-teneral males ; the numbers of tenerals of either sex are less accurate because they depend on the pupal population, reduction of which is delayed. In this paper reductions of the tsetse population refer to non-teneral females only (males were in fact more heavily reduced).

Two ways of measuring the results quantitatively were used. The " direct " method merely considers the drop in catches during a phase (its calculation is described below). If tsetse density were constant throughout the phase, the drop would be attributable to DDT-oxen. Examination of the past history of block 4 showed that at the Phase I time of the year density normally drops slightly from beginning to end. This drop will be superimposed on the artificial reduction. In Phase I, therefore, the " direct " method will give an overestimate of the effects of DDT-oxen. During Phase II, on the other hand, it was found that density normally rises ; in this phase the " direct " method will give an underestimate of reduction due to oxen.

The second (" control ") method of measuring results involves the difference between logarithms of catches in control and experiment blocks. This, it was found, is not constant through the year. Density in block 4 being normally higher, *log. (control catches)* were subtracted from *log. (block 4 catches)* throughout. The effect of the experiment was, of course, to lower this figure. Examination of records for the previous three years showed that during Phase I it normally rises slightly, making the " control " estimate for this phase too small. During Phase II, on the other hand, it normally drops, so that the Phase II " control " estimate will probably be too high.

We thus have the following expected biasses in the estimated results of the experiment : Phase I, " direct " too high, " control " too low ; Phase II, " direct " too low, " control " too high. When the results came to be calculated the expected overestimate exceeded the expected underestimate in each phase, and it was therefore considered reasonable to regard the arithmetic mean of both as the truest figure obtainable.

Calculation of percentage reduction.

Successive weekly catches of non-teneral females in block 4 differ insignificantly from a geometrical progression. There are theoretical reasons why this should be so. Their decline can therefore be expressed as r_w, the common ratio between weekly catches. Of those alive initially in any phase of the experiment, the proportion surviving in the kth and last week will be r_w^k, the proportion destroyed $1-r_w^k$, and the percentage destroyed $100 (1-r_w^k)$. r_w is best found, since all the observations are taken into account, by R. A. Fisher's abbreviated method of estimating the common ratio of a series of numbers in geometrical progression (Jackson 1939),

$$r_w = \frac{^w2 + {}^w3 + {}^w4 + \ldots \ldots {}^wk}{^w1 + {}^w2 + {}^w3 + \ldots \ldots {}^wk{-}1}$$

where $^w1$, $^w2$, etc., are successive weekly catches, k in all. In calculating percentage reductions this formula was used ; only catches made in the presence of oxen were included and, for convenience, week 14 was regarded as the end of Phase I and the beginning of Phase II. Fortnightly, instead of weekly, figures were entered in the formula for " control " estimates, giving r_f instead of r_w. These two quantities bear the relationship $\log. r_w = \frac{1}{2} \log. r_f$. Actual figures, not logarithms, were of course

used in " control " estimates ; they were antilogs. of log. differences, *i.e.*, catches in block 4 divided by those in the control. Table I gives values of r_w found in each phase of the experiment ; these numbers are in fact the average proportion of non-teneral females surviving from any one week to the next.

Interest also attached to r_p, the proportion of females surviving from one hunger-cycle to the next, or rather, to $1-r_p$, the proportion dying. Values of $1-r_p$ are also given in Table I, derived from

$$\log. r_p = \frac{\text{days in a hunger-cycle}}{\text{days in a week}}. \log. r_w$$

During Phase I the hunger-cycle was considered to be 4·2 days and during Phase II, 5 days.

TABLE I.

Calculated values of r_w and $(1-r_p)$.

			Phase I		Phase II	
			r_w	$1-r_p$	r_w	$1-r_p$
Direct Estimate	...	...	0·88	0·07	0·83	0·12
Control Estimate	...	...	0·94	0·04	0·78	0·16
Arithmetic Mean	...	...	0·91	0·06	0·80	0·14

r_w is the estimated proportion of non-teneral females surviving from any one week to the next. $(1-r_p)$ is the estimated proportion of non-teneral females dying from any one hunger-cycle to the next.

Estimation of extermination time.

" Extermination " was taken, rather arbitrarily, to be 99·99 per cent. destruction. At ordinary tsetse densities, this means the survival of one non-teneral female per $2\frac{1}{2}$ to 5 square miles, with smaller numbers of males and tenerals. It is possible that extermination might be reached with less than 99·99 per cent. destruction, but unlikely that a well-dispersed population could survive with more. The time required was calculated in weeks as $4\cdot0000/\log. r_w$, which assumes that destruction will go on at the same rate to extinction. The putative existence of density-dependent limiting factors in tsetse economy, and the possible selection of ox-avoiding strains, might render this assumption untrue. Since little is known about such factors. however, they can only be borne in mind when considering the results.

" Competition " (of cattle with game).

The proportion of females estimated to be feeding on oxen each hunger-cycle assuming, as is likely, that females contact oxen only when desiring to feed, is called for convenience " competition." The values shown in Table III were obtained from $1-r_p$ by means of an analysis too long for description here ; they are included because they indicate why destruction was slow. It is clear that they are of the right order, since if *all* females fed invariably on oxen nearly 70 per cent. (or, in Phase II, nearly 95 per cent.) would die each hunger-cycle ; whereas in Table I it is shown (values of $1-r_p$) that only 6 per cent. (or 14 per cent.) were in fact destroyed. Only a small proportion of females were therefore feeding on oxen.

Results.

Table II gives details of tsetse catches throughout the experiment, some of which are shown graphed in fig. 2. Table III summarises the main results.

Reduction of female G. pallidipes.

In Phase I these were reduced about 70 per cent. in 3 months. If this rate continued, extermination would take 22 months with approximate fiducial limits of

19 to 44 months. The time aimed at was 4 months, so destruction in this phase was about 1/6th the right speed.

It was calculated that the changes in Phase II should together produce a further reduction of 73 per cent. in 8 weeks, corresponding to an extermination time of $11\frac{1}{2}$ months, or a speed about 1/3rd of that required. Though this was still not good
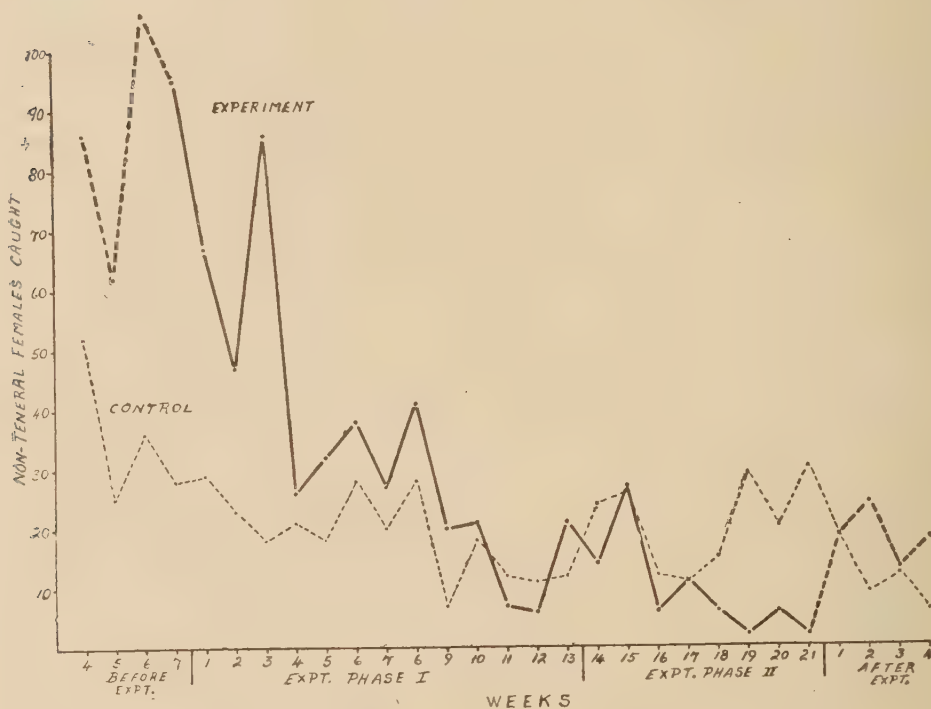
Fig. 2.—Weekly standard catches of non-teneral female tsetse from experiment and control blocks. Catches in the control block furnish only a partially adequate check on those in the experiment, since at the time of the year here shown density in the control block does not normally vary parallel with that in the experiment block.

enough, the experiment was continued in order to confirm that the essential factors governing its success had been understood. In Phase II a reduction of about 80 per cent. was in fact achieved, corresponding to an extermination time of about 9 months, or a speed rather better than 1/3rd of that required. A similar period under Phase I conditions would have produced only 48 per cent. reduction ; but the two results are not susceptible of statistical comparison, and it cannot be shown that they are significantly different. Splitting the herds and raising the killing-power probably both contributed to the improvement in Phase II, since, theoretically, neither alone could do so much.

Destruction appeared to take place evenly throughout the block, although block 4 contains areas of riverine thicket, hardpan and other well-differentiated sub-types of vegetation.

TABLE II.

Standard weekly catches from experiment and control blocks.

Week	G. pallidipes								G. swynnertoni	
	Non-Tenerals				Tenerals					
	Males		Females		Males		Females		Total catch	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
Before experiment										
1	76	51	43	25	24	11	12	15	9	220
2	122	57	62	22	13	8	12	7	20	162
3	154	101	90	31	42	24	´45	20	7	286
4	200	85	86	52	53	15	59	18	16	398
5	174	74	62	25	32	20	46	12	8	302
6	256	81	106	36	27	19	25	8	18	289
7	281	110	95	28	29	17	24	20	9	411
Experiment, Phase I										
1	173	96	67	29	28	23	45	20	7	426
2	150	55	47	23	27	13	35	7	8	358
3	152	53	86	18	53	7	39	10	4	394
4	48	50	26	21	21	9	24	11	4	412
5	44	58	32	18	14	9	28	6	2	327
6	67	64	38	28	22	10	29	13	2	413
7	48	56	27	20	32	7	20	11	4	446
8	34	53	41	28	21	3	23	7	2	406
9	33	38	20	7	20	7	21	5	2	426
10	26	33	21	18	15	6	7	1	3	424
11	21	31	7	12	8	8	5	4	2	311
12	17	22	6	11	4	4	9	12	1	347
13	11	40	21	12	6	1	6	6	0	417
Experiment, Phase II										
14	5	44	14	24	5	6	5	7	2	370
15	4	49	27	26	5	7	2	6	0	336
16	8	48	6	12	3	5	3	6	0	375
17	6	36	11	11	5	10	6	3	0	449
18	· 8	51	6	15	3	8	1	1	0	427
19	4	59	2	29	5	3	0	11	0	471
20	3	54	6	20	1 .	7	1	7	0	433
21	2	78	2	30	3	11	1	7	0	374
After experiment										
1	11	32	18	18	10	4	6	11	1	271
2	20	19	24	9	10	5	4	11	0	289
3	21	24	13	12	9	6	5	3	0	282
4	35	33	18	6	10	5	12	1	0	324

TABLE III.

Summarised results of the block 4 DDT experiment.

		Phase I	Phase II
Duration of experiment		13 weeks	8 weeks
Oxen in herds of		10	5
Weekly dosage pure DDT		9½ gm. per ox	19 gm. per ox
Monthly per square mile of bush { Total DDT used (c. 80 per cent. pp.)		11½ lb.	23 lb.
Total oil used		9½ gal.	19 gal.
Herdsmen		13	24
Mean kill		70 per cent.	95 (95) per cent.
Competition		13 ,, ,,	25 (20) ,, ,,
Reduction { in 13 weeks		70 ,, ,,	—
in 8 weeks*		48 ,, ,,	81 (73) ,, ,,
Extermination time in months		22	9 (11½)

*Under the meteorological conditions of Phase II " mean kill " is the proportion of flies which die after contact with treated oxen, ascertained by test. " Competition " is the estimated proportion of flies which fed on ox as opposed to game. " Reduction " is the estimated proportion of female flies which had been destroyed. " Extermination time " is the estimated time required to reduce females to 1/10,000th of their original numbers, assuming destruction proceeds at the same rate throughout. Figures in brackets under Phase II were predicted at the start of that phase.

Other effects on the tsetse population.

Since males are visibly more active than females they would be expected to decline quicker at first, as a result of more frequent contacts with oxen. Later, as they come to depend upon the number of female survivors, the number of males should decline in conformity with that of the females. This happened in both phases of the experiment as is shown by the figures in Table IV, which are derived from those in Table II.

TABLE IV.

Percentage of females amongst non-teneral flies.

		Experiment	Control
Before experiment : last 4 weeks		28	28
Phase I { first 5 weeks		33	26
next 4 weeks		41	27
last 4 weeks		41	30
Phase II { first 4 weeks		67	28
last 4 weeks		48	28
After experiment : first 4 weeks		47	29

Initially in each phase the percentage of females rises, because males are destroyed quicker ; later the percentage remains constant, the numbers of males being kept up by the better-surviving females. In the control the proportion is practically the same throughout.

During exposure to DDT-treated oxen the chances of surviving as long as usual will be lessened. A reduced mean age amongst non-teneral flies is therefore to be expected. Table V shows that this occurred. The figures are derived from measurements of wing-fray (Jackson, 1946) and are merely comparative (not absolute), since the fraying of wings with age has not been studied in detail for *G. pallidipes*. A natural lengthening of life probably occurred during Phase II as a result of the break of the drought, and accounts for the higher mean ages in this period.

TABLE V.

Mean age-index of non-tenerals in the experiment.

		Males	Females
Phase I	first 4 weeks	24	29
	next 4 weeks	15½	19
	last 4 weeks	15	18½
Phase II	first 4 weeks	13	24
	last 4 weeks	18	21
After experiment : first 4 weeks	24	24

These figures are indefinitely different from the true ages in days, but are comparable *inter se*. Both sexes live shorter average lives during the experiment, the shortening being greater in the case of males, which were destroyed more rapidly.

Teneral flies should become relatively more numerous compared with non-tenerals, partly because of the shortened life and partly because of the inevitable lag in reducing the subterranean pupal population. This was the case as is shown by the figures, derived from Table II, in Table VI.

TABLE VI.

Mean percentage of tenerals in catches.

	Experiment	Control
Before experiment : 7 weeks	19	21
Phase I : 13 weeks	31	20
Phase II : 8 weeks	30	15
After experiment : 4 weeks	29	23

The numbers of teneral flies depend on the numbers of pupae deposited about 1 month earlier. When destruction of adults takes place, as in the experiment, the proportion of tenerals rises to a level depending on the intensity of that destruction.

Effect of oxen on catches.

Tests showed that the presence of oxen immediately reduced catches significantly by something like one-third. This is clearly connected with the activity of the tsetse population. By calculating the experimental results only from catches in the presence of oxen, this factor is prevented from affecting the results.

After effects of the experiment.

As can be seen in Table II, removal of the oxen was followed by a rise in the numbers caught. This was too great to be accounted for entirely by the activity effect just mentioned. On theoretical grounds a small rise in population is to be expected on cessation of the experiment. There is some doubt, however, whether the two causes together could produce the observed rise in the catches. Possibly a natural fluctuation in population or activity took place at this time ; in any case, it does not seem that the experimental results can be seriously affected.

According to the evidence of routine fly-rounds carried out later, the population of *G. pallidipes* had practically returned to its normal level 3 months after stopping the experiment. It is necessary to suppose that both adults and pupae survived abnormally well during the recovery period to account for this. At present the only way this can conceivably happen is through a diminished action of density-dependent limiting factors. Although no evidence of the existence of such factors in holding up the predicted course of Phase II can be observed, it is nevertheless possible that they might slow down the late stages of destruction, making extermination a lengthier

matter than suggested above.   What this recovery does show beyond doubt, how
ever, is that such measures as those described may well be wasted unless they
proceed to extinction.

Discussion.

Reasons for the slow rate of reduction.

The results fell below expectation chiefly because of the small proportion of tsetse
which came to oxen—13 per cent. and 25 per cent. instead of 70 per cent., and 50
per cent., in the respective phases of the experiment, which would have given the
required speed.   Experiments showed that this was not due to repellence caused by
DDT.   It was partly due to the fact that *G. pallidipes* often feeds at night and in the
evening (Vanderplank, 1941 ; Chorley & Hopkins, 1942) when no oxen were in the
bush.   Using data from the authors just quoted it was calculated that probably
33 per cent. of *G. pallidipes* normally feed when oxen were not in the bush.   During
grazing hours, therefore, the proportion of tsetse contacting oxen must have been
much higher than 13–25 per cent.   It was not as high theoretically as the relative
numbers of large game and oxen would lead one to expect, which suggests that *G.
pallidipes* may prefer small game even in the presence of abundant larger hosts.

Visibility in block 4 was poor compared with that in normal thorn bush ;   the
effective range of attraction of the oxen might have been greater in the latter.   The
vegetation too was in full flush.   When the rains broke the ox kraals became inter-
mittently waterlogged and the oxen heavily fouled with mud, perhaps lessening their
effectiveness to tsetse.   Laboratory experiments have suggested that under humid
conditions tsetse are more resistant to the action of DDT than under dry conditions.
Some or all of these factors, all avoidable, may have played a part in reducing the
success of the experiment.

Possibilities of improvement.

As regards lethal power the treated oxen were almost maximally efficient during
Phase II ;   little advantage is to be gained by raising their killing power above an
average of 95 per cent.   The only possibilities of improvement lie in raising " com-
petition."   Some improvement was indeed achieved by splitting and dispersing the
herds, but the mounting cost of herdsmen precludes further splitting.   Similar
difficulties attend any attempt to lengthen the grazing hours.   There remains only
the possibility of increasing the relative density of oxen, either by using more or by
shooting out some of the game.   It appears, however, that with *G. pallidipes* small
game may be the chief competitors, and their destruction is a matter of some difficulty.
The gain to be achieved by raising the number of oxen is likely to be slight ;   a few
experiments tended to confirm the expectation that the number of tsetse attracted
varies as the square root of the number of oxen in the herd.   There is also an upper
limit to the number of oxen that thorn bush can support for any length of time.

Against *G. pallidipes* in a fairly typical habitat, therefore, this method of
eradication is likely to prove too lengthy and hence too expensive, but there is reason
to hope for more success against *G. morsitans* and *G. swynnertoni*.   The two latter
species are mostly inactive at night, inhabit more open country and tend to haunt
easily recognised feeding grounds (Jackson, 1930) in which the oxen might be expected
to attract a large proportion of the population.   In Phase I of the present experiment
the calculated extermination time of female *G. swynnertoni* is only 13 months as
against 22 months for *G. pallidipes*.

Summary.

An experiment to ascertain whether extermination of *Glossina pallidipes* could be
brought about by introducing large numbers of DDT-treated oxen into its habitat
was carried out in a small isolated block of bush in Tanganyika.

The oxen were sprayed with a solution of 9 per cent. w/v pure DDT and 9 per cent. w/v resin in groundnut oil.   It was estimated that when sprayed once weekly about 70 per cent. of tsetse settling on them were killed, and when sprayed twice weekly, about 95 per cent.   Oxen so treated were introduced in a numerical superiority of about 6 to 1 over the larger game in the bush, but were fewer than the small game.

After 3 months in which oxen sprayed once weekly were herded in tens in the bush for ten hours each day, the population of female tsetses was reduced by about 70 per cent.   After a further 2 months in which oxen sprayed twice weekly were herded in fives a reduction of 80 per cent. amongst the survivors was brought about.

It was calculated that if these rates of decline could be maintained extermination would take 22 months under the former conditions, and about 9 months under the latter.   There are reasons, however, for supposing that destruction might take place more slowly as extermination is approached.

It was shown that unless such a measure as this does proceed to extermination it may be largely wasted, for the tsetse population was able to recover its original numbers in a relatively short time after decimation.

The conditions governing the success of this type of measure against tsetse are discussed.   Reasons are given for supposing that quicker destruction of *G. pallidipes* is unlikely to be economically attained ;   on the other hand, it is considered that greater success might well be achieved against *G. morsitans* or *G. swynnertoni*.

Acknowledgements.

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THE PERSISTENT TOXICITY UNDER STANDARDISED FIELD CONDITIONS OF PYRETHRUM, DDT AND "GAMMEXANE" AGAINST PESTS OF STORED FOOD.

E. H. N

By A. F. O'Farrell, B.Sc., B. M. Jones, B.Sc. and G. A. Brett, B.Sc., A.R.C.Sc.

Pyrethrum sprays in a heavy oil base, such as those described by Potter (1935, 1938) are now widely used in Britain for the control of insects attacking stored foodstuffs. Practice has not yet been greatly influenced by the introduction of "DDT" (2, 2 *bis* (*p-chlorophenyl*) 1, 1, 1 trichlorethane) and "Gammexane" (the gamma isomer of benzene hexachloride), since the direct application of these insecticides to foodstuffs is officially discouraged in this country on the grounds of possible hazards to consumers. Nevertheless, the well-known persistent properties of these synthetic insecticides may have considerable value in the treatment of storage premises, a purpose for which they have already been widely used abroad.

The toxicity of DDT to various stored products insects has been investigated by many workers in the laboratory and in the field, including Cotton and others (1945), Davis (1946) and Swingle & Mayer (1944). "Gammexane" has been less widely studied ; its general properties were discussed by Slade (1945), and it is known to possess exceptionally high toxicity to *Calandra* spp. In general, however, published information on the persistent toxicity of insecticides against stored products insects under field conditions is scanty, and even for pyrethrum such information is surprisingly scarce. It would be rash to predict the relative toxicities of pyrethrum, DDT, and "Gammexane" against stored products insects under field conditions from the published data, although laboratory comparisons have been made between pyrethrum and DDT against warehouse insects at the D.S.I.R. Pest Infestation Laboratory.

Laboratory comparisons are often somewhat unsatisfactory from a practical standpoint, owing to the wide variety of types of surface which have to be treated in spraying a warehouse. Although widely divergent results are obtained with such practically important substrates as concrete, brick, wood, and various types of sacking, under both laboratory and field conditions, these have been little studied. Potter (1938) found that pyrethrum-in-oil films on wooden fruit boxes remained effective for at least a week against *Ephestia elutella*, Hb., under British field conditions, whereas Nel and Mathew (1944) found that similar films on a paper substrate lost toxicity very rapidly against the same species under warehouse conditions in South Africa. Hewlett and Parkin (1947) investigated persistent films of pyrethrum in P31 oil against *Tribolium castaneum*, Hbst., on a variety of building materials under laboratory conditions, and suggested "pre-treatment" of such surfaces before spraying (see also Hewlett & Parkin, 1945). Apart from work on stored products insects, important comparisons of insecticidal deposits on wood and other surfaces against bedbugs (*Cimex lectularius*) were made by Barnes (1945) who found DDT superior both to "Gammexane" and pyrethrum, the toxicity of the latter being negligible after only three weeks. Madden, Lindquist and Knipling (1944), on the other hand, reported a high kill of the same species after 22 days with a deposit of only 2·5 mgm. pyrethrins per square foot ; they stated, however, that the deposit was ineffective later, whereas DDT continued to give complete mortality, even after scrubbing, at 78 days.

As a result of the inadequacy of published information on the practical effectiveness and persistence of insecticidal deposits on most of the surfaces likely to require treatment in warehouses, and in view of certain field observations by the

authors, the present work was initiated. Its object was defined as obtaining practical information, under standardised field conditions, on the persistent toxicity of several types of insecticidal deposits on a variety of surfaces likely to be encountered in treating a food warehouse. The surfaces were to be treated by the standard field technique, but with some refinements (*e.g.*, deposits were to be measured, etc.) ; this was considered to provide a closer approach to practical conditions than the use of closely controlled deposits obtained in a spray tower. Toxicities were to be estimated by observing mortality in batches of selected test insects after standard exposures on the treated surfaces, and the surfaces themselves were to be kept under suitable warehouse conditions throughout the experiment.

Site of the Experiments.

Observations were carried out in a disused basement in an old wharf on the south bank of the Thames. Conditions here were certainly damper than in most London food warehouses, but this disadvantage was offset by the certainty that treated surfaces would be left undisturbed throughout the period of observations, and by the relative uniformity of the physical conditions. Improvised benches were set up along the walls of the experimental room, which, with its concrete floor, roughly whitewashed brick walls, concrete ceiling, wooden doors, and dilapidated windows below street level, letting in draughts but a minimum of daylight, was fairly typical of basement storage in the older warehouses, although disuse and bomb damage combined to render it inferior to most food storage premises. Frequent whirling hygrometer readings taken throughout the period of observation (September, 1946, to January, 1947) showed a complete absence of violent fluctuations in temperature and humidity. The former showed a steady downward trend, with minor fluctuations (never greater than 3°C. and seldom more than 1·5°C. in amplitude) from a maximum of 15°C. in September to a minimum of 5°C. in January, when the experiment was discontinued ; humidity was very constant, remaining within the limits 90-96 per cent. R. H. throughout, except for a brief fall to 80–85 per cent. R.H. during early October.

In this room the treated surfaces were exposed to " warehouse conditions " during the period of observations. To avoid accidental contamination of the experimental room, all insecticidal treatments were carried out in outbuildings separated from it by a wide open yard. This precaution made it impossible to use the concrete floor of the experimental room for observing the toxicity of deposits on concrete, but a site suitable for this purpose was found on the concrete floor of a large room, at the opposite end of the yard, used for storage of infested empty bags awaiting fumigation.

Surfaces treated and Insecticides used.

Seven materials, representing surfaces likely to be frequently encountered in warehouse spraying, were selected for investigation, and a suitable number of " units " of each material, approximately 1 foot square, were prepared, as follows :—

Building materials.

(i) Concrete.—Squares were marked out *in situ* on the floor.
(ii) Brickwork.—Special units were made up, each of three bricks laid on one another and joined with cement mortar, to represent the surface of an inside brick wall.
(iii) Wood.—Squares of smooth unpainted deal 1 in. thick were used.

Food containers.

(i) Cotton flour bag ... ...
(ii) Jute flour bag ... ...
(iii) Heavy hessian grain sack ...
(iv) Light hessian grain sack ...

Squares of the appropriate size were cut from bags obtained from a sack merchant.

The insecticides used were also seven in number, as follows :—

(*a*) 1·3 per cent. pyrethrins in P31 oil, the standard warehouse spray used by the Ministry of Food for general purposes.

(*b*) 0·8 per cent. pyrethrins in P31 oil, the spray usually employed by the Ministry of Food for moth control in warehouses.

(*c*) 5 per cent. commercial DDT (containing approximately 70 per cent. of the pure parapara compound) dissolved in commercial kerosene.

(*d*) 1 per cent. DDT suspension in water, made up trom a proprietary wettable powder containing 5 per cent. DDT.

(*e*) 5 per cent. commercial DDT in talc, used as a dust.

(*f*) 1 per cent. "Gammexane" in commercial kerosene, made up by dilution of a concentrate containing 10 per cent. purified "Gammexane" in cyclohexanone.

(*g*) A "Gammexane" dust, consisting of 4 per cent. crude benzene hexachloride in kaolin (gamma isomer content 0·5 per cent.).

Kerosene was selected as a base for the DDT and "Gammexane" sprays in preference to P31 oil primarily because it was considered that the characteristic properties of these insecticides would be better shown by using a volatile carrier ; moreover, the solubility of DDT in P31 oil was known to be rather low, and it was desired to use materials corresponding as closely as possible with ordinary commercial formulae. The use of a watery suspension of DDT was prompted by commercial reports of its successful use against *Calandra* and other pests in empty maltings, while the dusts were investigated mainly on account of their potential practical value in circumstances where spraying might be found inconvenient or expensive.

Application of Insecticides to Surfaces.

The standard procedure adopted was to place one unit of each material to be treated (*i.e.*, six units in all) on a sheet of wall board laid upon an elongate "target", approximately 9 ft. long by 1 ft. wide, marked out on the floor of the outbuilding where treatment was carried out. The whole set of units was then sprayed or dusted, and at once removed, as a whole, on the board, to one of the benches in the experimental room. After cleaning equipment, etc. and ventilating the spray room to ensure that it was clear of spray mist or floating dust particles, a fresh set of units was put down on a new sheet of board and the process repeated with the next insecticide on the list, until units of all selected materials except concrete had been treated with each insecticide. (The watery DDT suspension was not applied to the four types of "sack" however, since a water-based spray would not be used on food containers in practice.)

Treatment of concrete was carried out a few days after the other surfaces ; as it occupied one large room in a separate building, the only possible precaution against contamination of one insecticide by another was to have the areas selected for treatment as widely separate as possible ; this led to the elimination of 0·8 per cent. pyrethrum in P31 oil and the two dusts, as their inclusion would have caused overcrowding of the limited space available.

Spraying of all surfaces with the various insecticides was carried out, for the sake of uniformity, by the same operator, using a standard Ministry of Food spraying equipment consisting of a De Vilbiss "HM" oil-spray gun supplied with compressed air through 60 feet of ¾-in. air hose by a petrol-driven ("Aeraspray C.P.5.") compressor set to 75 lbs. per square inch. The container of the gun was removed, however, and the dip tube connected by glass and rubber tubing to a burette, thus permitting measurement of the delivery of insecticide. The actual spraying was carried out by

sweeping the gun smoothly to and fro over the target, about 18 ins. above the sprayed surfaces ; the required time of spraying being roughly predetermined, for the particular nozzle setting in use, by preliminary trial sprayings with the appropriate spray-base. The nozzle setting used for the P31 oil sprays approximated to that generally recommended in practice for film spraying, *i.e.*, a nett delivery of rather more than 1 gallon per hour. With other spray bases, the nozzle was adjusted until the spray emitted was judged by eye to be of the right type. Before proceeding from one insecticide to the next, the gun, burette, and tubing were dismantled and thoroughly cleaned ; the next insecticide was then sprayed through the apparatus three or four times before proceeding to the actual treatment of the next batch of material. With the dusts, which were applied by means of " acme " bellows-type hand distributors, similar general procedure was followed, and a separate distributor was used for each dust.

TABLE I.

Insecticides and surfaces, showing deposits of active toxicant (Pyrethrins, DDT, or " Gammexane ") in milligrams per square foot for each combination of insecticides and surface.

Insecticides	Surfaces						
	Concrete	Brick	Wood	Cotton	Jute	Hessian (heavy grain sack)	Hessian (light bran sack)
1·3 per cent. pyrethrins in P31 oil	25·35	21·12	16·9	12·675	12·675	12·675	12·675
0·8 per cent. pyrethrins in P31 oil	Not treated	10·4	7·8	7·8	5·2	5·2	7·8
5 per cent. DDT in kerosene	65·0	145·0	145·0	72·5	72·5	72·5	72·5
1 per cent. DDT suspension in water	104·0	162·0	117·0	Not treated	Not treated	Not treated	Not treated
5 per cent. DDT/talc dust	Not treated	48·75	56·8	97·5	97·5	118·75	118·75
1 per cent. " Gammexane " in cyclohexanone and kerosene	16·25	45·5	47·0	48·75	42·25	42·25	30·9
0·5 per cent. " Gammexane " (as 4 per cent benzene hexachloride/ kaolin dust ...	Not treated	11·78	11·78	9·75	9·75	6·5	6·5

Rough measurements of deposits were made by exposing on each unit surface during treatment a glass slip, of standard area, which was weighed in a bottle immediately before and after exposure. The results so obtained (*i.e.*, weight of spray or dust deposited on a known area) were converted into milligrams of active toxicant per square foot on the basis of the strengths of insecticides used. These figures, for each combination of surface and insecticide, are shown in Table I. Some of the glass slips were accidentally displaced or contaminated by dirt during exposure, so that the figures given are based on less than one slip per surface ; the table has thus been constructed on the apparently reasonable assumption that deposits on neighbouring surfaces were sufficiently uniform to justify taking mean figures where necessary. As might be expected, the DDT/talc dust was the most erratic deposit, but deposits of all materials varied considerably. This was probably due in part to differences in relative position of the units along the path of the spray swathe, and

in part to fundamental differences in the degree of rebound of particles from the various types of surface. The deposits on concrete were high with pyrethrum and low with other insecticides, probably as a result of the separate treatment of this material. Taken as a whole, Table I provides a basis for comparison and, in all probability, an indication of the variation in deposits likely to occur in practice even with the work of a single skilled operator.

Biological Observations.

Persistent toxicities of the 42 different combinations of insecticide and surface investigated were assessed by placing on each, at intervals, batches of " test insects ". confined under an inverted circular glass dish 3 ins. in diameter and $\frac{1}{2}$ in. deep. (These dishes had to be sealed on to the brick and concrete surfaces by rings of plasticine, but on other surfaces the weight of the dish was sufficient to prevent the escape of insects.) Three hours was adopted as the standard period of exposure for the test insects, which were then collected into numbered tubes and put aside to " recover " for not less than 24 and not more than 48 hours. Insects exposed on untreated " control " surfaces received exactly the same treatment. At the end of the " recovery " period, each batch was examined and the mortality recorded. Insects " knocked down " (*i.e.*, alive but too severely paralysed to walk) were counted as " dead", since it was found that under the conditions of the experiment those which had not recovered sufficiently to walk after 24 hours in a clean tube seldom did so later ; the upper limit of 48 hours for the " recovery period " was set to avoid undue mortality of controls with relatively short-lived insects such as adult *Ephestia kühniella*.

Selection of test insects was necessarily governed primarily by their ready availability in large numbers over the whole period of the experiment. This severely limited the choice, but those selected were known from field observations to represent a wide range of reactions to pyrethrum films under practical conditions, ranging from high resistance to extreme susceptibility. They were as follows :—

Calandra granaria, L., and *C. oryzae*, L., adults, obtained from mixed cultures kept by the Ministry of Food.

Tribolium castaneum, Hbst., adults, from a culture kindly provided by the D.S.I.R. Pest Infestation Laboratory, Slough.

Ephestia kühniella, Zell., larvae (full-grown) collected from debris in a London rice mill.

E. kühniella, Zell., adults, collected in the field as required.

The cultures of *Calandra* spp. and *T. castaneum* were kept in a heated office above the experimental rooms ; a large bag of heavily infested debris from the mill was kept in a cupboard adjoining the experimental room and provided a continuous supply of full grown larvae of *E. kühniella*. Adult *E. kühniella* were collected the day before each observation, in batches, in 3×1 in. specimen tubes, and were left in the tubes in the experimental room overnight. The rice mill was an excellent source of supply at first, but was later fumigated, moths being subsequently obtained from a large flour mill.

From these sources insects required for each set of observations were collected in batches of five to ten, in clean 3×1 in. glass specimen tubes with numbered corks. Each batch was quickly tipped out on to the required surface and covered with a glass dish ; the empty numbered tube was left on the dish, its number and that of the corresponding deposit being recorded, so that insects were returned to the same tubes after exposure and subsequent mortality recorded under the tube numbers. Owing to the flight activity of *E. kühniella* adults and the web-spinning behaviour of their larvae, observations on them had to be made separately from one another and from the *Calandra* spp. and *T. castaneum* ; the latter, however, were easier to handle and

were generally exposed together. At the end of the exposure, the larvae and adults of *E. kühniella* were removed with forceps and suction tubes were used for the beetles. The extremely low mortality of controls (even *E. kühniella* adults) throughout the observations indicated that this handling was not harmful.

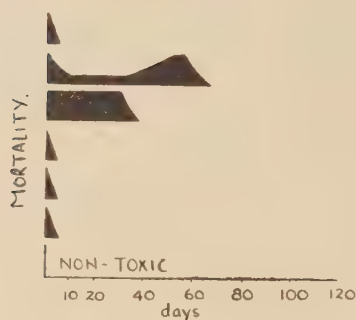Calandra granaria (L.) and Calandra oryzae (L.)

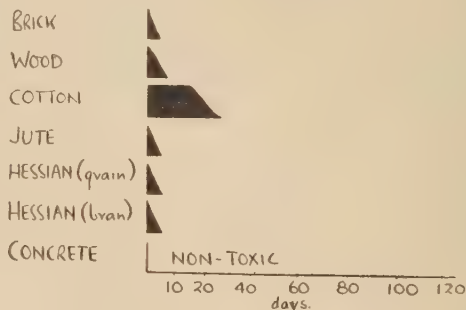Fig. 1. 1·3% PYRETHRINS/P31 OIL

Fig. 2 0·8% PYRETHRINS/P31 OIL

Fig. 3. 5% DDT/KEROSENE
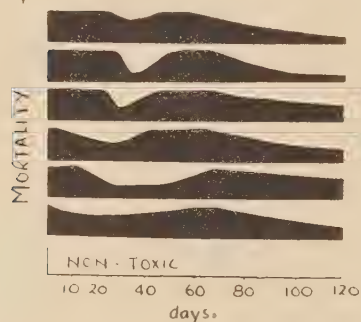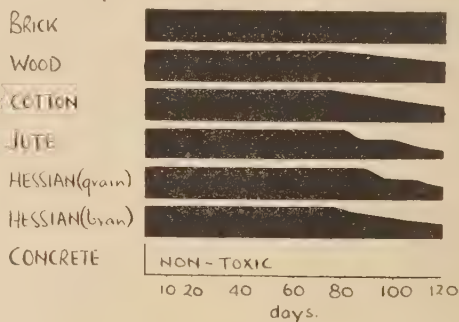
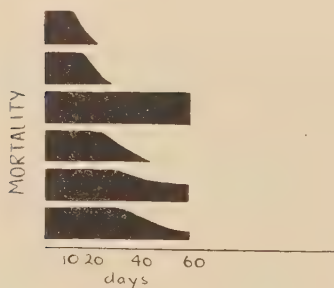Fig. 6 1% GAMMEXANE/CYCLOHEXANONE/KEROSENE.

Fig. 4. 5% DDT/TALC
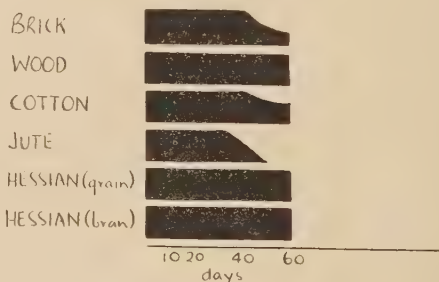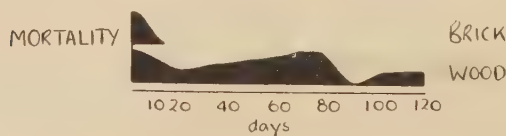
Fig. 7. 0·5% GAMMEXANE/TALC

Fig. 5. 1% DDT/WATER SUSPENSION

Exposures of all four test insects thus involved three separate sets of observations. Other commitments made it impossible to lay down a rigidly set interval between observations on each species, but these were made as often as possible and necessary, working out in practice to an interval of 5–7 days during the first month and 10–15 days thereafter, up to the 80th day. No further observations were made until the final series was carried out about the 120th day. Observations on the DDT and " Gammexane " dusts were terminated at the 60th day, owing to accidental destruction of the deposits by water entering through a leak in the roof. The use of *T. castaneum* was discontinued after 65 days owing to the exhaustion of available material.

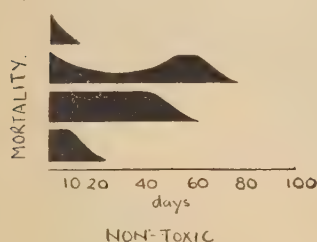Tribolium castaneum (Herbst.)

Fig 8 1·3% Pyrethrins/P.31 Oil

Fig 9. 0·8% Pyrethrins/P.31 Oil.
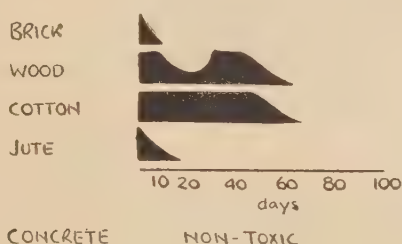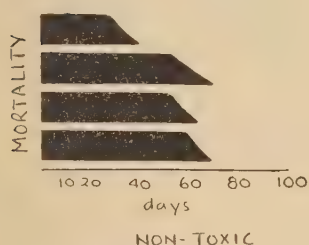
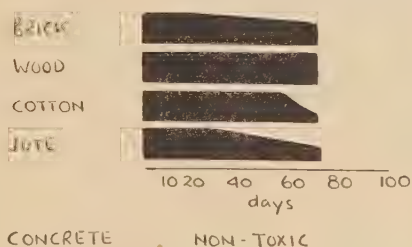Fig.10. 5% DDT/Kerosene

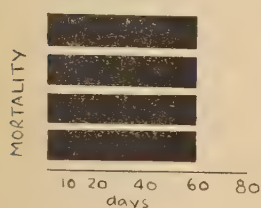Fig 13 1% Gammexane/Cyclohexanone/Kerosene

Fig 11. 5% DDT/Talc

Fig 14 0·5% Gammexane/Kaolin
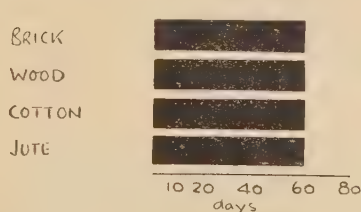
Fig 12 1% DDT/Water Suspension
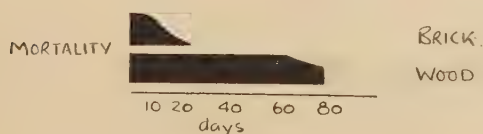
The results are presented in the form of 26 block diagrams (figs. 1–26) each of which shows, graphically and in comparable form, the mortality observed for one insecticide and test insect on all types of surface investigated. Collectively, the series of diagrams provides a comparison of mortality for all combinations of insecticide, surface, and test insect. This form of presentation has been adopted as better suited to the present work than direct tabulation of the large mass of figures representing the original data. Diagrams for the untreated control surfaces are not given, because mortality of control insects was negligible throughout, being limited to the very occasional death of one of a batch.

Ephestia kuhniella (Zell.) larvae

Fig. 15. 1·3% PYRETHRINS / P.31 OIL   Fig. 16. 0·8% PYRETHRINS / P.31 OIL

Fig. 17. 5% DDT / KEROSENE   Fig. 18. 1% GAMMEXANE / CYCLO   Fig. 19. 0·5% GAMMEXANE
           HEXANONE / KEROSENE.   / KAOLIN,

5% DDT / TALC
1% DDT / WATER SUSPENSION   }   ALL SURFACES NON-TOXIC.
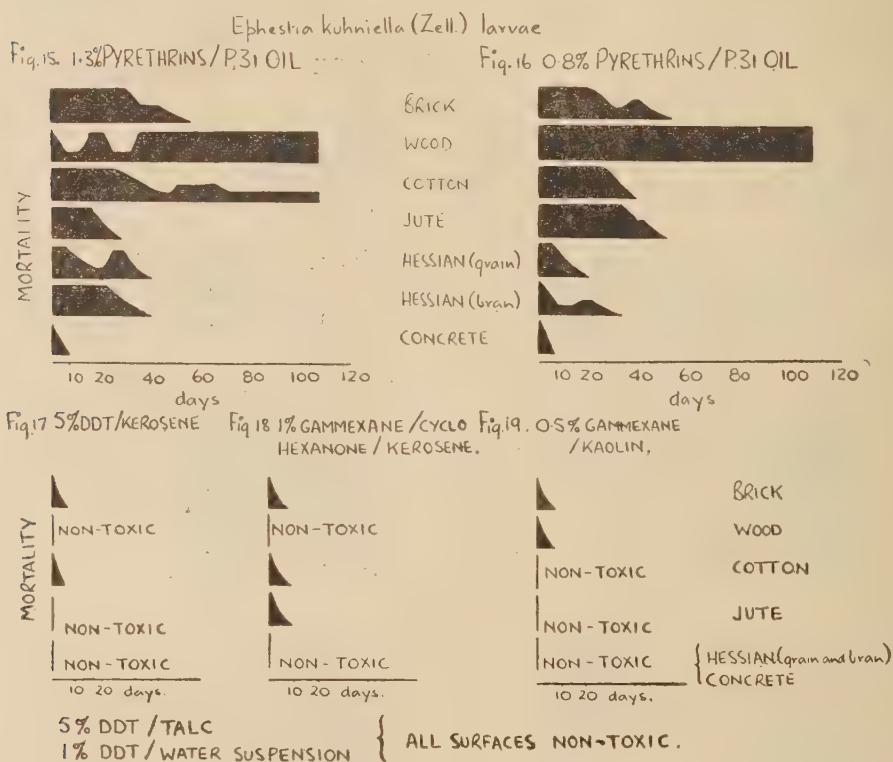
To summarise these results of greatest practical importance, Table II has been constructed to show the age in days of each deposit on each surface at the last occasion on which complete mortality was recorded for each species. Concrete is omitted because none of the deposits on this material was highly toxic even at the first observation. Otherwise, figures are given for all combinations of insecticide, surface, and test insect. It should be explained that the figure " O " does *not* denote zero mortality, but merely that complete mortality was never recorded on that particular deposit. Reference to the block diagrams will show what level of mortality occurred in such cases. A " + " sign denotes that the figure quoted is a minimum, since mortality was still complete at the time of the last observation. An asterisk(*)

appearing with figures for some pyrethrum and DDT deposits indicates that fluctuations in toxicity occurred, so that low mortalities recorded at one or more observations were succeeded by a return to complete mortality, often lasting for some time (see figs. 1, 3, 8, 9, 15).   These results will be further discussed later.

Ephestia kuhniella (Zell.) adults

Fig 20. 1·3% PYRETHRINS / P31 OIL

Fig 21 0·8% PYRETHRINS / P31 OIL.

Fig.22. 5% DDT / KEROSENE
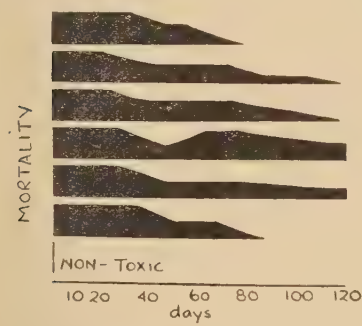
Fig 25. 1% GAMMEXANE / CYCLOHEXANONE / KEROSENE
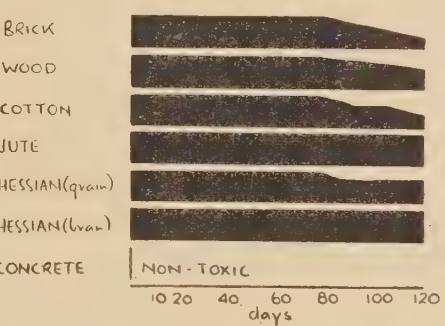

Fig 23. 5% DDT / TALC.

Fig 26 0·5% GAMMEXANE / KAOLIN

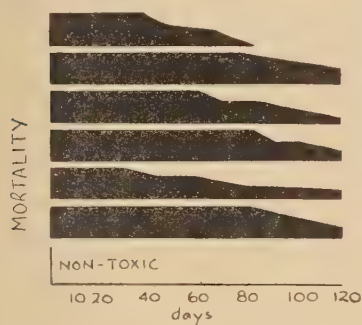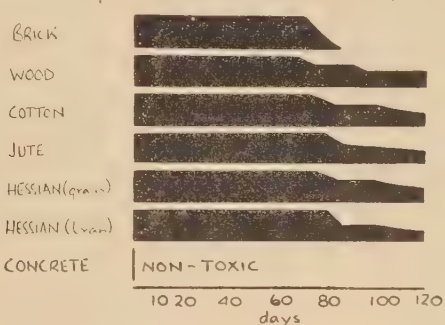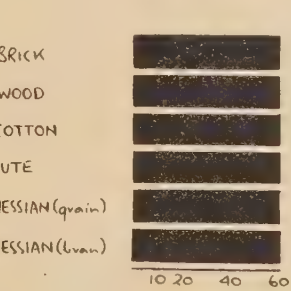Fig. 24. 1% DDT / WATER SUSPENSION.
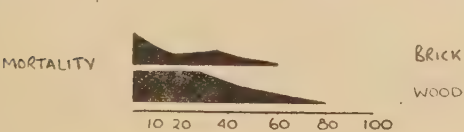
TABLE II.

Maximum observed ages of insecticidal deposits giving 100 per cent. mortality on various surfaces.

Insecticides used	Age, in days, of deposits at latest observations of complete mortality of exposed insects																							
	Brick				Wood				Cotton bag				Jute bag				Hessian (heavy grain bag)				Hessian (light grain bag)			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Test insects ... ... 1·3 per cent. pyrethrins in P31 oil ...	0	0	30	39	0	0	112*†	80	21	35	30	60	0	0	10	80	0	NR	0	30	0	NR	15	80
0·8 per cent. pyrethrins in P31 oil ...	0	0	14	60	0	29*	70†	80	20	35	25	80	0	0	30	80	0	NR	15	80	0	NR	0	80
5 per cent. DDT in kerosene	62*	25	0	35	62*	49	0	21	62*	49	0	21	62*	65†	0	21	62*	NR	0	21	62*	NR	0	30
1 per cent. DDT suspension in water ...	0	0	0	0	70*	60†	0	30	(N.B.—Since a water suspension could never be used on these surfaces in practice, no observations were considered necessary.)															
5 per cent. DDT/Talc dust	11	60†	0	30	18	60†	0	10	60†	60†	0	21	25	60†	0	21	25	NR	0	30	25	NR	0	15
1 per cent. "Gammexane" in cyclohexanone and kerosene	120†	28	0	80	72	65†	0	80	72	49	0	80	72	29	0	120†	72	NR	0	80	72	NR	0	120†
0·5 per cent. "Gammexane" (as 4 per cent. of benzene hexachloride/kaolin) dust	32	60†	0	60†	60†	60†	0	60†	33	60†	0	60†	33	60†	0	60†	60†	NR	0	60†	60†	NR	0	60†

A=*Calandra* spp. B=*Tribolium castaneum.* C=*Ephestia kühniella* larvae. D=*Ephestia kühniella* adults.
*Unreliable figure (see text p. 142).
†100 per cent. mortality recorded up to end of observations.
N.R. No observations.

Discussion of Results.

So far as comparison between insecticides is concerned, the most striking feature of the results is the remarkably persistent toxicity shown by pyrethrum films against *E. kühniella* adults and larvae. Pyrethrum was clearly more toxic than DDT and about equal to "Gammexane" against the adults, and was the only one of the three insecticides showing high toxicity to the larvae. (The low toxicity of DDT to larvae of moths which, as adults, are quite susceptible has already been observed, *e.g.*, by Davis, 1946.) Results with *Calandra* spp. and *T. castaneum* for the various insecticides are in line with published work, although the toxicity of pyrethrum films on wood and cotton surfaces was decidedly more persistent than expected. It is probable that the conditions of the experiment were very favourable to the chemical stability of pyrethrins. Certainly the adverse influence of light (see Tattersfield, 1932, Nel and Mathew, 1944, Lindquist and others, 1946) was absent, but darkness should also have favoured the toxicity of DDT (Lindquist and others, 1946), which in fact appeared inferior to pyrethrum against *E. kühniella*, the only test insect which was highly susceptible to both insecticides. As for "Gammexane", apparently the best insecticide of the three for general purposes (apart from its ineffectiveness against *E. kühniella* larvae), all results have to be viewed with caution owing to its volatility. Conditions for insects confined, even under a porous material, on a "Gammexane" deposit are likely to be exceptionally rigorous, since in these circumstances they are exposed to an appreciable concentration of the toxic vapour, which would be absent or negligible on a well-ventilated surface in the open. We are unable to suggest any practical means of overcoming this experimental difficulty, since any simple form of artificial ventilation would lead to equally unreliable results arising from the violent reaction of insects to draughts. Accordingly, our conclusion in regard to "Gammexane" is that final assessment of its value can be made only by extensive and accurate observation of its effectiveness in the field.

The importance of the type of surface in determining effectiveness of treatment was clearly shown by the results for concrete. The film of pyrethrins in P31 oil lost toxicity very rapidly on this absorbent material (compare the observations of Hewlett & Parkin, 1947). It is possible that emulsions might be of value in treating such surfaces. Robinson (1943) showed that deposits of pyrethrum were more toxic to ticks, on an artificial "plaster" substrate, when applied as emulsions of P31 oil in water than in the oil alone. Results on concrete with the two liquid preparations of DDT were also unsatisfactory : both with the kerosene solution and the watery suspension the deposits were admittedly somewhat low, but only slightly less than those on some of the other surfaces which proved far more toxic. It is probable that ineffectiveness on concrete also characterises the "Gammexane" spray ; however, through an unfortunate miscalculation, the deposit was very much lower than elsewhere and no firm conclusion can be drawn from its low toxicity. Nevertheless, where concrete has to be treated it is believed that dusts would prove superior to most types of spray.

As already indicated, the practical results for surfaces other than concrete are summarised in Table II. Study of these figures reveals that the persistent toxicity of pyrethrum-in-oil films was greatly affected by the nature of the surface treated, whereas the deposits of DDT and "Gammexane" derived from evaporation of kerosene-based sprays gave tolerably uniform results on all types of surface. Similar uniformity characterised the results with "Gammexane"/kaolin dust and, to a lesser extent, those with DDT/talc dust. The water suspension of DDT, on the other hand, was virtually useless on brick, but equal to or better than the kerosene spray on wood. So far as pyrethrum films are concerned, the following is a list, based on the results as a whole, of the order of suitability of surfaces as substrates for a persistently toxic film, viz. : 1, wood ; 2, cotton flour bag ; 3, jute flour bag ; 4, light hessian bran sack ; 5, heavy hessian grain sack ; 6, brickwork (see figs. 1, 2,

8, 9, 15, 16, 20, 21). The difference between Nos. 1 and 2 on this list and the rest is very considerable, mainly because cotton is the only substrate on which pyrethrum deposits were observed to be persistently toxic to *Calandra* spp., while films on wood maintained high toxicity against *E. kühniella* adults and larvae for an amazingly long period.

The film of 1·3 per cent. pyrethrins on wood, however, was erratic in its behaviour during the first few weeks (fig. 15) only " settling down " about the 35th day, to give complete mortality of *E. kühniella* larvae until the end of the observations. These fluctuations in mortality did not occur with adult *E. kühniella* on the same deposit, nor with adults or larvae on the 0·8 per cent. film. Similar fluctuations did occur, however, with *Calandra* spp. and *T. castaneum* on wood (figs. 1, 8, 9), possibly due to the individual variation in susceptibility of these insects, but such an explanation does not indicate why the fluctuations were confined to deposits on wood, nor does it seem at all appropriate to the results observed with *E. kühniella* larvae.

A further point of interest in relation to the pyrethrum deposits was the relatively high toxicity of the 0·8 per cent. film as compared with the 1·3 per cent. ; the results suggest that initial pyrethrins content, within the limits 0·8 per cent. to 1·3 per cent., was a relatively unimportant factor in determining the persistent toxicity of deposits under the conditions of the experiment.

Fluctuation in mortality was also observed with *Calandra* spp. on the DDT deposits from the kerosene spray, on all treated surfaces (fig. 3). This was probably caused by changes in toxicity due to formation of crystals in the deposit (see Parkin & Green, 1945). Presumably the toxicity of the deposits was adequate to give complete mortality of *T. castaneum*, but not the more resistant *Calandra* spp., until the postulated physical changes took place. Rather similar behaviour was seen with the watery DDT suspension, on wood (figs. 5, 12), but not with any of the DDT dust deposits (figs. 4, 11). Some minor fluctuations in mortality of *E. kühniella* adults on the various DDT deposits (figs. 22–24) also occurred, but these were so slight and irregular that they can probably be ascribed to accidental variations.

" Gammexane " deposits were characterised by their general high and uniform toxicity. A point of special interest is that the dust deposits (figs. 7, 14, 19 and 26), seemed quite as toxic as the deposits derived from the kerosene spray (figs. 6, 13, 18 and 25) so far as the observations went ; yet reference to Table I shows that the quantity of " Gammexane " present was several times greater for the spray deposits than for the dusts. The dust, of course, contained the other isomers of benzene hexachloride, which were absent from the spray, but the toxicity of such isomers is said to be negligible compared with that of the gamma isomer. One is led to conclude that the physical state of the " gammexane " deposit from the spray (which, it will be remembered, was a dilution with kerosene of a cyclohexanone concentrate, and not merely a solution of the solid in kerosene) was such as to render it less toxic than the dry material present in the dust.

Summary.

The persistent toxicity of films of pyrethrum in P31 oil may endure on suitable surfaces under warehouse conditions for much longer periods than hitherto supposed, but unexplained fluctuations in toxicity may occur.

Within the range 0·8 per cent. to 1·3 per cent. total pyrethrins, the pyrethrum content appears to be only a minor factor in determining the persistent toxicity of such films.

The nature of the surface sprayed is of great importance in determining the persistent toxicity of pyrethrum films in P31 oil, which is negligible on concrete

and increases steadily through the following list of surfaces—brick, heavy hessian, light hessian, jute, cotton, and wood ; the last-mentioned two being particularly good substrates.

Residual deposits of DDT or " Gammexane " derived from kerosene sprays are of little use on concrete, but appear otherwise little affected by the nature of the surface treated ; they give a uniform degree of persistent toxicity on the various surfaces listed.

The treatment of concrete by dusts is suggested. Otherwise no particular practical advantage attaches to the use of DDT or " Gammexane " in dust form, except that the toxicity of the latter may be higher as a dust than as a spray ; the persistent toxicity of dusts is not apparently influenced by the nature of the substrates.

There is no apparent advantage, other than non-inflammability, attached to the use of DDT in a watery suspension instead of an oil spray.

Both DDT and " Gammexane " are toxic to adults but practically non-toxic to larvae of *Ephestia kühniella* (and probably other warehouse moths), which are better controlled by pyrethrum films that are effective against larvae as well as adults.

" Gammexane " appears to be somewhat more effective as a stored products insecticide than DDT but more field experience is required to establish this.

Acknowledgements.

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STUDIES ON WEST AFRICAN FOREST MOSQUITOS.—PART I. THE SEASONAL DISTRIBUTION, BITING CYCLE AND VERTICAL DISTRIBUTION OF FOUR OF THE PRINCIPAL SPECIES.

By P. F. MATTINGLY.

(From the *Yellow Fever Research Institute, Lagos, Nigeria.*)*

The studies described below were carried out between 7th June 1945 and 13th June 1946 at a field station near the village of Itowolo on the left bank of the Ogun river about three miles up-stream from the point at which it flows into Lagos Lagoon (Southern Nigeria) (fig. 1). The vegetation in this area consists of moderately dense swamp forest with an average canopy height of between 50 and 60 feet, the latter figure appearing to be rarely exceeded except by occasional large cotton trees (*Ceiba pentandra*). The ground is intersected by numerous small creeks and the field st tion itself stood on a small island. At certain times of the year the creeks become choked with water lettuce (*Pistia*) and at such times they form an ideal breeding place for *Taeniorhynchus africanus*, Theo., *Anopheles hargreavesi*, Theo., and other species of mosquitos. Lagos Lagoon is fringed with mangrove. The site was chosen in the belief that it would yield a large and varied mosquito population and in the event this proved to be the case. Over 30,000 mosquitos were taken in 23 catches and the population comprised more than 50 species. The principal object of the work was the accumulation of detailed information regarding the seasonal occurrence, biting cycle and vertical distribution of these mosquitos and for this purpose a continuous 24-hour catching technique was employed similar to that used by Bates (1944), and Haddow & others (1947).

Details of Technique.

Catching was carried out by adult Africans working in pairs on platforms at ground level, 22 feet, 40 feet and 52 feet. All the platforms were situated in a single tree (*Cynometra* sp.) and were numbered in the above order I, II, III and IV, respectively. The Africans worked stripped to the waist and with their legs bare and were changed from one platform to another every two hours. The first catch was largely of an experimental nature and was used to obtain a rough estimate of the relative attractiveness to mosquitos of individual Africans. The pairs were then arranged so that each should as far as possible be equally attractive and they were subsequently maintained unchanged except where illness or other causes necessitated a substitution. During the first 16 catches the allocation of the various pairs to platforms was made at random but after this it was planned so that no boy should have been on any particular platform during any particular hour an undue number of times. In view of the large possibilities of error due to the human factor in a technique of this kind it was thought better to use the boys in a random manner than to run the risk of imposing an artificial periodicity on the figures by alternating them regularly. The mosquitos were caught by the boys on themselves and their partners in small test tubes which were plugged with cotton wool and placed in linen bags each capable of holding a hundred. The bags were collected once an hour and each was provided with a label showing the time at which it was collected and

the platform from which it came. Identification followed and by maintaining a rigid routine it was possible to obtain an hour to hour record of the biting activity and vertical distribution of each species. Hourly temperature and humidity readings were made on each platform with sling psychrometers. Self-recording thermographs
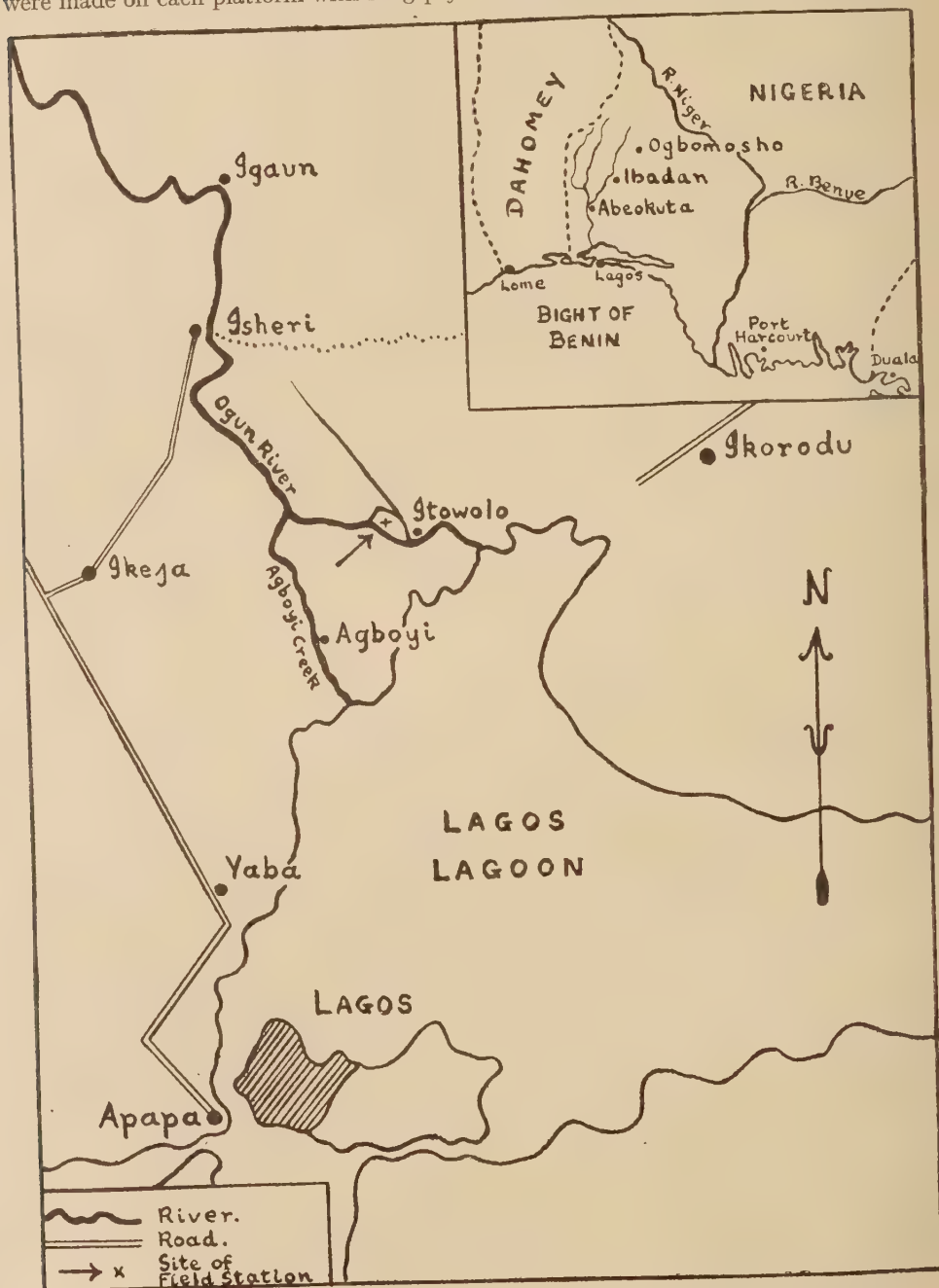

Fig. 1.—Sketch map of the Ogun River study area with (inset) map of Southern Nigeria.
Scale : Ogun River map, 1 inch=4·75 miles. S. Nigeria 1 : 16,000,000 (approx.).

were used during the later catches but their rate of change was found to be too slow to give satisfactory readings during the daytime and they were therefore used only for a few hours during the night. In order that the mosquitos should be collected as nearly as possible at the same time, the bags were picked up rapidly while ascending the tree and the psychrometer readings were taken in a more leisurely fashion while coming down. Additional temperature and humidity figures together with records of wind speed, wind direction and barometric pressure were obtained from the meteorological station at Ikeja airfield about four miles west of the catching station and daily rainfall figures were obtained from the same source. Unfortunately no suitable apparatus was available for obtaining regular light readings, although on two occasions readings were taken with a small photographic exposure meter, and recourse was had to the tables of sunrise, sunset, beginning and ending of morning and evening twilight and moonrise and moonset given by the Nautical Almanac in order to obtain a general picture. Approximately one half of all the mosquitos taken were triturated and injected into mice in the hope of recovering yellow fever virus but without success although the technique was subsequently shown to be satisfactory when virus was recovered from three batches of *Aëdes aegypti*, L., taken in houses during an outbreak of the disease at Ogbomosho.* Altogether 23 continuous 24-hour catches were made. The first of these has been omitted from the records given below as catching had to be suspended for four hours on account of heavy rain. For subsequent catches each platform was provided with a roll of canvas which could be stretched overhead to form a temporary roof when necessary. Quite high biting rates were obtained even during heavy rain. Each catch was begun at 15.15 hrs. Local Mean Time (16.00 hrs. Nigerian Standard Time) and continued until 15.15 hrs. L.M.T. the following day. From 18.15 hrs. until 05.15 hrs. L.M.T. each platform was lit by a single hurricane lamp. The camp site associated with the field station lay about 100 yards west of the tree and here the bush was cleared over an area sufficient to accommodate two tents. Lighting was kept to a minimum, not more than two or three hurricane lamps being used at any one time.

The Mosquito Population.

In the present paper only four of the more abundant species taken at Itowolo are discussed. The remainder will be dealt with in a later communication. The total numbers taken on each platform are shown in Table I. The figures given refer to females only. In addition to these the total catch included 3 males of *Anopheles gambiae*, Giles, 2 of *Aëdes africanus*, Theo., and 450 of *Taeniorhynchus africanus*. No males of *Anopheles hargreavesi*, Theo., were taken. In general no attempt was made to separate *A. gambiae* from *A. melas*, Theo., as it does not appear possible at present to separate individuals of these species on adult characters. Some 40 specimens taken at different hours and on all four platforms during a single catch were submitted to Dr. R. C. Muirhead Thomson who succeeded in obtaining eggs from 12 of them. These proved without exception to be *A. melas* and it is believed that the Itowolo population contains a high proportion of this species. The test cannot, however, be regarded as conclusive since it may indicate a differential survival rate in the catching tubes, brackish water breeding species being, in the author's experience, particularly favoured in this respect. It is probable also that the seasonal distribution of the two species would vary.

Rainfall and Seasonal Distribution.

Weekly rainfall figures and monthly totals from Ikeja for the period 1st May, 1945 to 17th June, 1946 are given in Table II. Fig. 2 shows a curve based on the monthly totals superimposed on the seasonal distribution curves of the species under discussion. The latter are based on average monthly catches the figures for which

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* A paper dealing with this outbreak by Drs. J. C. Bugher, F. N. Macnamara, Mrs. M. Taylor and the author is at present in preparation.

TABLE I.

Hourly totals of each species taken during 22 24-hour catches at Itowolo.

Hour ending	A. gambiae					A. hargreavesi					T. africanus					A. africanus				
	Platform IV	III	II	I	Total	Platform IV	III	II	I	Total	Platform IV	III	II	I	Total	Platform IV	III	II	I	Total
16.15	0	0	0	2	2	0	0	0	4	4	0	0	1	9	10	0	1	13	3	17
17.15	0	0	0	0	0	0	0	1	1	2	0	0	1	9	10	0	4	9	5	18
18.15	0	0	0	0	0	0	0	4	1	5	3	6	5	26	40	5	8	15	5	33
19.15	3	12	45	69	129	5	12	62	69	148	60	207	364	371	1,002	61	30	19	2	112
20.15	2	6	20	21	49	9	34	106	58	207	53	210	452	461	1,176	27	4	6	1	38
21.15	4	12	34	18	68	13	60	128	160	361	73	216	449	684	1,422	8	7	5	0	20
22.15	5	17	42	48	112	19	56	134	188	397	94	280	385	808	1,567	15	11	3	0	29
23.15	10	23	40	81	154	24	49	205	235	513	69	277	514	1,055	1,915	17	11	2	0	30
00.15	4	21	61	94	180	21	80	337	239	677	108	270	457	920	1,755	16	6	3	1	26
01.15	9	25	80	88	202	24	90	251	221	586	82	302	408	966	1,758	12	11	0	0	23
02.15	7	25	97	113	242	19	76	281	303	679	102	244	333	918	1,597	8	6	1	0	15
03.15	15	29	84	124	252	24	60	267	315	666	77	227	361	756	1,421	8	5	1	0	14
04.15	10	43	138	180	371	19	70	288	322	699	86	187	396	826	1,495	22	6	5	0	33
05.15	31	67	160	161	419	29	72	217	301	619	114	169	292	713	1,288	5	4	1	0	10
06.15	5	14	61	105	185	6	42	144	517	709	25	92	140	441	698	15	10	4	1	30
07.15	1	0	2	41	44	1	4	19	158	182	9	4	30	163	206	3	15	3	1	22
08.15	0	0	0	19	19	0	2	4	82	88	1	5	11	161	178	0	0	2	5	7
09.15	0	0	0	6	6	0	0	3	49	52	0	0	10	175	185	2	1	5	3	11
10.15	0	0	2	17	19	0	0	3	39	42	1	0	5	153	159	0	0	2	1	3
11.15	0	0	1	5	6	0	1	3	14	18	0	0	4	58	62	0	0	6	3	9
12.15	0	0	1	19	20	0	0	1	12	13	1	2	0	64	67	0	0	2	2	4
13.15	0	0	0	13	13	0	1	0	6	7	0	1	0	40	41	0	2	3	0	5
14.15	0	0	0	5	5	0	0	0	10	10	0	0	2	75	77	0	0	7	4	11
15.15	0	0	0	5	5	0	2	0	14	16	0	0	0	46	46	0	1	2	2	5
Total	106	294	868	1,234	2,502	213	711	2,458	3,318	6,700	958	2,699	4,620	9,898	18,175	224	143	119	39	525

are taken from Table III. The curves are discontinuous since no catches were attempted in December and January when it was judged that the majority of species would be in abeyance and the catches would be " saturated " with *T. africanus*. The low average recorded for *A. gambiae* and *Aëdes africanus* in June, 1946 is clearly due to the fact that the last catch was made less than half-way through the month and emphasises the very limited peak period occurring in these species.   *Anopheles hargreavesi* and *T. africanus*, being in part dependent on river level and accretions of *Pistia* from up-stream, are less immediately affected by local conditions.
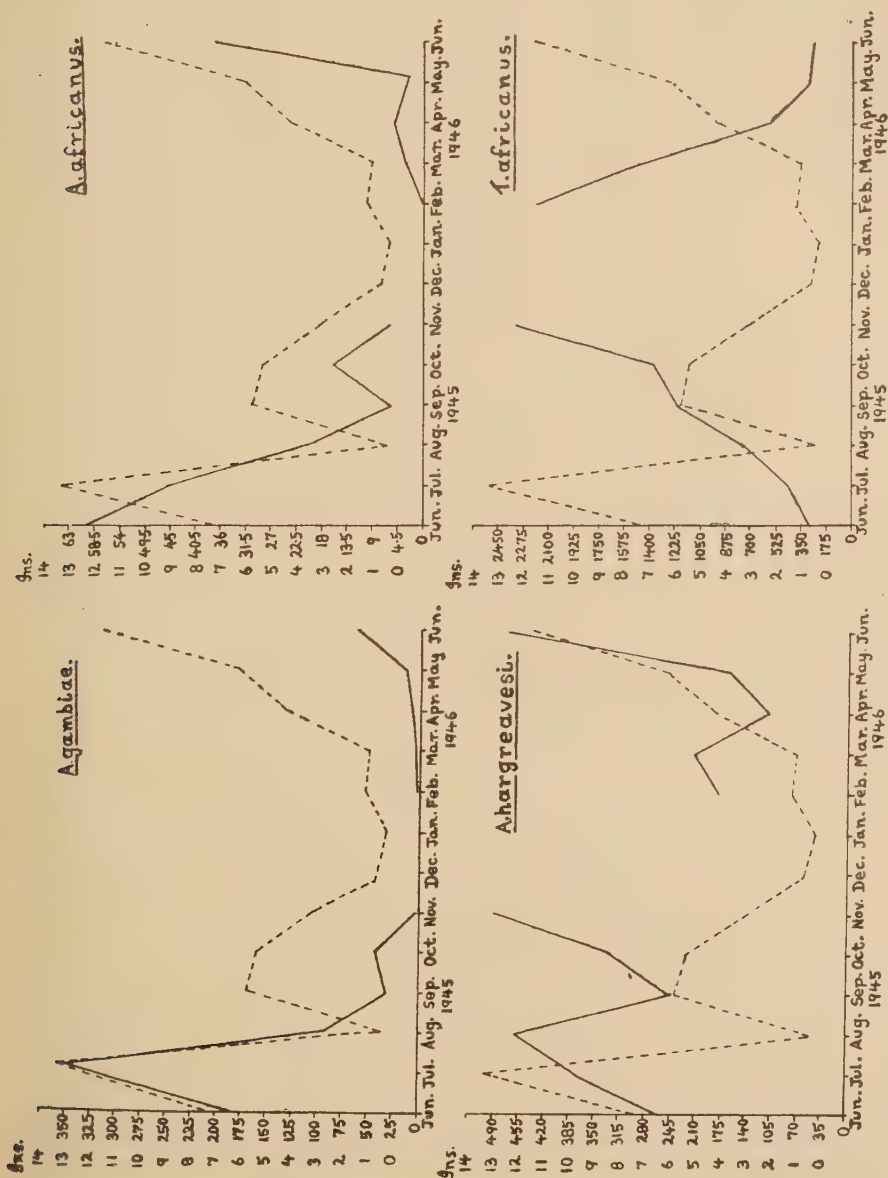
Fig. 2.—Seasonal distribution at Itowolo.   (Rainfall at Ikeja shown by broken line.)

TABLE II.
Rainfall at Ikeja (inches).

Month	Week ending	Rain (inches)	Monthly total	Month	Week ending	Rain (inches)	Monthly total
1945				**1945**			
May	7th	0·65		Dec. ...	3rd	0·45	
	14th	Trace	3·92		10th	0·00	
	21st	0·90			17th	0·70	0·70
	28th	2·19			24th	0·00	
June	4th	0·19			31st	0·00	
	11th	4·32	7·40	**1946**			
	18th	0·34		Jan. ...	7th	0·00	
	25th	0·53			14th	Trace	
July	2nd	3·47			21st	0·36	0·36
	9th	4·60			28th	0·00	
	16th	5·19	13·36	Feb. ...	4th	0·00	
	23rd	Trace			11th	0·29	1·20
	30th	2·29			18th	0·00	
Aug.	6th	0·31			25th	0·91	
	13th	Trace	0·43	Mar. ...	4th	0·00	
	20th	0·00			11th	0·94	1·02
	27th	0·00			18th	0·07	
Sept.	3rd	0·53			25th	0·01	
	10th	0·78	5·80	Apr. ...	1st	0·00	
	17th	1·15			8th	1·97	
	24th	0·46			15th	0·00	4·27
Oct.	1st	3·45			22nd	0·34	
	8th	1·67			29th	1·96	
	15th	0·81	5·40	May ...	6th	0·96	
	22nd	1·37			13th	1·65	6·07
	29th	0·55			20th	2·50	
Nov.	5th	1·50			27th	0·96	
	12th	0·33	3·10	June ...	3rd	2·30	
	19th	0·88			10th	4·48	11·55
	26th	0·50			17th	3·76	

TABLE III.
Seasonal Distribution of Mosquitos at Itowolo.

Month	Date	Catch No.	A. gambiae		A. africanus		A. hargreavesi		T. africanus	
			No. caught	Monthly average	No. caught	Monthly average	No. caught	Monthly average	No. caught	Monthly average
1945										
June ...	14–15	2	73		49		174		362	
	21–22	3	353	188	54	60	242	260	245	290
	28–29	4	137		76		363		264	
July ...	5–6	5	387		44		177		135	
	12–13	6	375	350	48	45	344	375	371	450
	19–20	7	259		63		466		571	
	26–27	8	377		24		513		722	
Aug. ...	2–3	9	66		23		316		371	
	9–10	10	176	92	34	21	235	459	597	757
	23–24	11	34		6		827		1,304	
Sept. ...	6–7	12	54	32	1	6	220	243	611	1,208
	20–21	13	10		11		265		1,804	
Oct. ...	4–5	14	83	43	9	16	193	329	963	1,383
	24–25	15	3		22		464		1,803	
Nov. ...	21–22	16	1	1	6	6	488	488	2,312	2,312
1946										
Feb. ...	13–14	17	3	3	0	0	181	181	2,177	2,177
Mar. ...	13–14	18	4	4	3	3	212	212	1,481	1,481
Apr. ...	3–4	19	11		2		51		432	
	17–18	20	4	8	7	5	169	110	743	588
May ...	1–2	21	5		1		71		186	
	15–16	22	25	15	5	3	256	164	434	310
June ...	12–13	23	62	62	37	37	473	473	287	287
		Total	2,502	—	525	—	6,700	—	18,175	—

Temperature and Humidity.

Table IV shows the average temperatures and saturation deficits recorded on all platforms during the last 21 catches. Complete figures were not obtained for the first two catches. The curves shown in fig. 3 are based on 2-hourly averages of the four readings from the separate platforms. It will be seen from the table that during the early afternoon a temperature inversion takes place between the second and third platforms. This is due to the sunlight falling obliquely through a gap in the canopy. Haddow (1945) has made a detailed study of the microclimate in relation to biting activity in Bwamba County, Uganda which lies at the opposite extremity of the
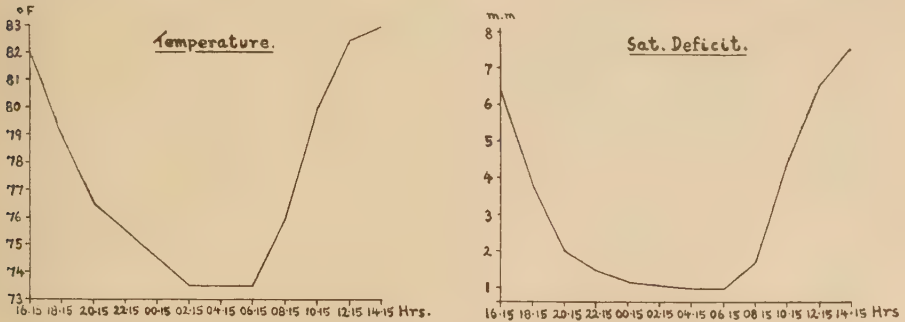

Fig. 3.—Temperature and Humidity at Itowolo.

TABLE IV.

Average Temperatures and Saturation Deficits at Itowolo.

| Time (L.M.T.) | Temp. (Degrees Fahrenheit) | | | | | Sat. Deficit (Mm. Hg.) | | | | |
| | Platform | | | | Av.* | Platform | | | | Av.* |
	I	II	III	IV		I	II	III	IV	
16.15	80·5	81·5	82·5	82·0	81·5	4·2	5·7	6·3	6·2	5·6
17.15	79·0	80·0	80·0	80·0	80·0	3·4	4·5	4·8	5·0	4·4
18.15	77·5	78·0	78·0	78·0	78·0	2·2	2·9	3·4	3·4	3·0
19.15	76·5	77·0	77·0	77·5	77·0	1·8	2·3	2·3	2·5	2·2
20.15	75·5	76·0	76·5	76·5	76·0	1·4	1·8	2·1	2·3	1·9
21.15	75·0	75·0	75·5	76·0	75·5	1·4	1·3	1·7	1·8	1·6
22.15	74·5	75·0	75·5	75·5	75·0	1·1	1·3	1·6	1·6	1·4
23.15	74·5	74·5	74·5	75·0	74·5	1·0	1·3	1·4	1·3	1·3
00.15	74·0	74·0	74·0	74·5	74·0	0·9	1·2	1·4	1·2	1·2
01.15	73·5	74·0	74·0	73·5	73·5	0·9	1·2	1·4	1·0	1·1
02.15	73·0	73·5	73·5	73·5	73·5	0·8	1·0	1·1	1·0	1·0
03.15	73·5	73·0	73·5	73·0	73·5	0·9	1·0	1·1	0·9	1·0
04.15	73·5	73·0	73·5	73·0	73·5	1·1	1·1	1·2	1·0	1·1
05.15	73·0	73·0	73·5	73·5	73·5	1·0	1·1	1·0	1·0	1·0
06.15	73·0	73·0	73·5	73·0	73·5	0·8	0·9	1·0	0·9	0·9
07.15	74·5	75·0	75·0	75·0	75·0	0·9	1·3	1·3	1·2	1·2
08.15	76·5	77·0	77·5	77·5	77·0	1·5	2·2	2·6	2·4	2·2
09.15	78·5	79·5	80·0	80·5	79·5	2·6	3·7	4·4	4·6	3·8
10.15	80·0	80·5	81·5	81·5	81·0	3·6	5·1	5·8	6·1	5·1
11 15	81·0	82·0	82·0	82·5	82·0	4·9	6·2	6·4	6·7	6·1
12.15	82·0	83·5	83·0	84·0	83·0	5·6	7·5	7·7	8·0	7·2
13.15	82·5	83·5	83·0	84·0	83·5	6·4	8·0	7·6	8·2	7·6
14.15	82·5	83·5	83·0	84·0	83·0	6·3	7·8	7·9	8·2	7·6
15.15	82·0	82·5	83·0	83·5	82·5	6·2	7·2	7·2	7·9	7·1

*It is to be noted that these figures are not the averages of those given for the individual platforms. They are the averages of the four-platform averages for individual catches, the latter being taken to the nearest 0·5°F. or 0·1 mm. Hg. before averaging.

Lower Guinean Forest district of the West African zoogeographical sub-region. His temperature range appears to have been somewhat lower and more restricted. Unfortunately he gives detailed figures only for one week in June and one week in September so that detailed comparisons are not possible. At Itowolo over the whole year the temperature ranged from 68·5°F. to 92·0°F. and the saturation deficit from 0·0 mm. to 16·0 mm. The coolest month was August, 1945 (68·5°F. to 86·0°F.) and the hottest April, 1946 (73·0°F. to 92·0°F.). Psychrometer readings were taken to the nearest 0·5°F.

Times.

The times given in Table IV and all times given throughout are Local Mean Time. In the case of Itowolo this is 13·8 minutes ahead of Greenwich Mean Time. For practical purposes it has been taken to be 15 minutes ahead so that mosquitos collected exactly on the hour by Nigerian Standard Time (one hour ahead of G.M.T.) are shown as having been collected at 15 minutes past the previous hour by L.M.T. The figures based on Local Mean Time are directly comparable with those given by Haddow for similar experiments in Uganda.

Light.

As previously stated no apparatus was available for regular light readings and the information given by the Nautical Almanac was used for obtaining a general picture. The data obtained from this source, corrected for latitude, are given in Table V. At Itowolo the times of sunrise and sunset vary between about 05.45 and 06.20 and 17.40 and 18.20 hrs., respectively, but it is to be noted that for the relatively

TABLE V.

Times of Sunrise, Sunset, Twilight, Moonrise and Moonset at Itowolo.

Catch No.	Astro. twilight began	Sun rise	Sun set	Astro. twilight ended	Moon rose*	Moon set*	Moon's age at mid-night**
2	04.28	05.45	18.15	19.32	09.43	22.24	3·8
3	04.29	05.46	18.17	19.33	15.00	03.09	10·8
4	04.31	05.48	18.18	19.34	21.03	09.11	17·8
5	04.33	05.50	18.19	19.34	02.46	15.30	24·8
6	04 35	05.52	18.19	19.36	08.23	21.02	2·4
7	04.37	05.53	18.20	19.35	13.40	01·46	9·4
8	04.40	05.53	18.19	19.33	19.48	07.59	16·4
9	04.40	05.55	18.17	19.32	01.34	14.21	23·4
10	04.42	05.54	18.16	19.28	07.04	19.40	1·0
11	04.43	05.54	18.11	19·22	18.30	06.43	15·0
12	04.42	05.52	18.04	19.14	05.46	18.18	29·0
13	04.41	05.50	17.57	19.06	17.08	05.23	13·4
14	04.37	05.47	17.49	18.59	05.14	17.37	27·4
15	04.36	05.47	17.42	18.53	21.04	09.56	17·8
16	04.38	05.53	17.40	18.54	19.46	08.41	16·0
17	05.06	06.17	18.12	19.22	15.56	04.47	10·8
18	04.58	06.08	18 11	19.21	14.47	03.35	9·2
19	04.47	05.57	18.09	19.19	07.04	19.38	0·8
20	04.41	05.52	18.08	19.18	19.08	07.15	14·8
21	04.35	05.46	18.08	19 20	05.37	18.18	28·8
22	04.30	05.44	18.09	19.23	17.50	05.56	13·4
23	04.28	05.45	18.15	19.32	16.33	04.37	12·1

*That part of the moon's cycle is given which covered the night of the catch in question. Thus on the occasion of the second catch the moon rose at 09.43 hours on the morning of the day on which the catch was begun while on the occasion of the ninth catch it rose at 01.34 hours on the day on which the catch was ended.

**In days.

TABLE VI.
Meteorological Data from Ikeja.

Catch No.	Time	Bar. Pressure (mbs.)	Wind Speed (m.p.h.)	Wind Direc- tion	Catch No.	Time	Bar. Pressure (mbs.)	Wind Speed (m.p.h.)	Wind Direc- tion
2	17.15	1013·1	0	—	9	05.15	1014·4	6	N
	20.15	1013·6	0	—		08.15	1015·5	10	WSW
	23.15	1013·8	2	SW		11.15	1014·1	6	SW
	02.15	1013·3	0	—		14.15	1012·6	6	WSW
	05.15	1014·4	0	—	10	17.15	1012·9	6	WSW
	08.15	1015·8	2	SW		20.15	1013·8	2	SW
	11.15	1014·5	6	WSW		23.15	1014·1	0	—
	14.15	1013·5	6	SW		02.15	1013·8	0	—
3	17.15	1010·9	6	SW		05.15	1014·6	0	—
	20.15	1013·4	6	SW		08 15	1015·6	2	SW
	23.15	1013·1	0	—		11.15	1015·0	10	WSW
	02.15	1012·4	0	—		14.15	1013·8	10	WSW
	05.15	1012·6	2	WNW	11	17.15	1013·1	2	WSW
	08.15	1012·9	2	SW		20.15	1013·5	6	SW
	11.15	1013·3	10	SW		23.15	1014·3	6	WSW
	14.15	1012·7	6	SSW		02.15	1013·5	2	SW
4	17.15	1013·3	6	SW		05.15	1013·7	0	—
	20.15	1014·2	0	—		08.15	1015·2	2	WSW
	23.15	1014·6	2	SW		11.15	1014·1	10	SW
	02.15	1014·5	2	SW		14.15	1012·7	10	SW
	05.15	1014·6	2	SW	12	17.15	1014·4	2	SW
	08.15	1015·3	10	WSW		20.15	1016·3	6	SW
	11.15	1014·3	10	SW		23.15	1016·5	6	SW
	14.15	1012·7	10	WSW		02.15	1016·4	6	SW
5	17.15	1012·8	6	WSW		05.15	1015·6	6	SSW
	20.15	1013·8	2	SW		08.15	1016·4	10	SW
	23.15	1013·5	6	NE		11.15	1016·1	6	SW
	02.15	1012·6	0	—		14.15	1015·0	6	WSW
	05.15	1013·3	2	NNE	13	17.15	1014·0	6	SSW
	08.15	1014·9	2	SW		20.15	1016·2	0	—
	11.15	1014·7	6	SW		23.15	1015·6	2	SW
	14.15	1013·7	6	SW		02.15	1014·3	2	SW
6	17 15	1014·1	6	WSW		05.15	1014·9	0	—
	20.15	1015·1	0	—		08.15	1016·6	2	SW
	23.15	1015·0	6	SW		11.15	1015·0	6	WSW
	02.15	1014·0	6	SW		14 15	1013·6	6	WSW
	05.15	1015·1	2	SW	14	17.15	1010·7	2	S
	08.15	1015·9	6	W		20.15	1011·9	2	SSW
	11.15	1014·6	10	WSW		23.15	1011·3	0	—
	14.15	1013·3	6	SW		02.15	1011·5	0	—
7	17.15	1013·6	2	SW		05.15	1012·4	2	SE
	20.15	1014·0	0	—		08.15	1012·8	6	SW
	23.15	1014·4	0	—		11.15	1012·4	2	SW
	02.15	1013·8	0	—		14.15	1011·5	6	SW
	05.15	1014·6	0	—	15	17.15	1011·2	6	SSW
	08.15	1015·6	6	WSW		20.15	1012·3	6	SW
	11.15	1014·8	6	SW		23.15	1012·6	2	S
	14.15	1013·4	6	WSW		02.15	1012·0	0	—
8	17.15	1013·1	10	WSW		05.15	1012·5	10	NW
	20.15	1013·9	6	SW		08.15	1012·9	6	SW
	23.15	1014·6	6	SW		11.15	1012·0	6	SW
	02.15	1013·8	6	WSW		14 15	1010·7	10	SW
	05 15	1014·2	0	—	16	17.15	1008·5	6	N
	08.15	1014·1	10	WSW		20.15	1009·5	0	—
	11.15	1014·5	10–15*	WSW		23.15	1009·8	6	SW
	14.15	1014·0	10–15*	WSW		02.15	1008·9	2	SE
9	17.15	1012·5	6	SW		05 15	1009·8	0	—
	20.15	1013·6	2	SW		08.15	1010·2	6	N
	23.15	1013·7	0	—		11.15	1009·0	6	ENE
	02.15	1013·6	0	—		14.15	1008·3	10	SSE

*Gusty.

restricted periods during which the majority of species are present in significant numbers the variation is much smaller. In considering the possible relationship between any particular biting curve and the times of sunrise and sunset it is therefore necessary to ascertain, from the figures for seasonal distribution, during which catches the species in question was abundant. The times given in Table IV for the beginning of morning and ending of evening twilight refer to astronomical twilight (sun 18° below the horizon).

Other environmental factors.

Meteorological data obtained from Ikeja airfield are given in Table VI. They were obtained only for the first 16 catches but as the main bulk of the mosquitos were taken during these catches they are considered adequate for a general picture. The effect of wind speed and wind direction on the total numbers of mosquitos taken in individual catches might be expected to be most pronounced in the case of *Anopheles gambiae* and *A. melas*. In these species the flight range is considerable and, especially in the case of *melas*, the principal breeding places were probably some distance from the catching station and part of the flight would have had to be made over open country. The local effect of these factors would probably have been most marked on the top platform. Studies on the concentration of atmospheric carbon dioxide in a Southern Nigerian forest were made by Evans (1939) but he gives figures only for the hours of daylight. No measurements have as yet been made at Itowolo but there can be little doubt that they would be of considerable interest.

Effects of rain.

Heavy rain was encountered on a number of occasions but it was not sufficiently frequent or prolonged to affect the over-all figures. In general its effect on biting activity appeared to be slight. During the 13th catch it occurred between 17.45 and 19.15 hrs. and might have been expected to affect *Aëdes africanus* which has a marked biting peak between 18.15 and 19.15 hrs. On the occasion in question only 11 of this species were taken but for the time of year this figure appeared to be high rather than low and the biting cycle (3 during the hour ending 19.15, 2 during the hour ending 20.15 and the remainder one at a time during the night) and vertical distribution appeared normal. During the 14th catch heavy rain fell between 03.10 and 05.30 hrs. when it might have been expected to affect *Anopheles gambiae*. In fact the behaviour of this species differed from normal in the occurrence of an unusually early biting peak at 03.15 hrs., apparently as a result of some depression in biting activity during the normal peak period. On this occasion the total figures from all four platforms for the three hours ending 03.15 to 05.15 inclusive were 17, 12 and 9, respectively. In the case of *A. hargreavesi* also a reduction in biting activity was noticed which might be attributed to heavy rain. This species has a morning biting peak which normally occurs between 05.15 and 06.15 hrs. On the occasion in question heavy rain fell between 05.35 and 06.15 hrs. and during this hour only 8 *A. hargreavesi* were taken out of a total catch of 344. During the following hour 17 were taken and this was the only occasion on which the total for the period 06.15 to 07.15 hrs. exceeded by a significant amount that for the period 05.15 to 06.15 hrs. *T. africanus*, a species which showed high biting activity throughout the night and is usually to be taken in small numbers during the day, appeared to be quite unaffected by heavy rain whenever it fell.

Biting Cycles.

The precise implications of the term " biting cycle " will be discussed in a later communication. For the present it may be taken to imply the hour to hour change in the numbers of any particular species taken during the course of a standard 24-hour catch. Of the species taken at Itowolo 15 were judged to be sufficiently abundant to give a significant picture. As previously stated 22 full 24-hour catches were made

so that for the more abundant species 22 separate biting cycles are available while for the remainder the number of biting cycles obtained was equal to the number of catches in which each occurred. To obtain a general picture and to facilitate comparison all the biting cycles available for each species have been combined to give a mean biting cycle characteristic of that particular species. Separate biting cycles were, of course, obtained for each of the four platforms making it possible to obtain a picture of the hour to hour variation in vertical distribution but in the tables which follow the figures from all four platforms have been combined to give an over-all figure.

TABLE VII.

Biting Cycles of *T. africanus* and *A. hargreavesi* at Itowolo.

Time	T. africanus				A. hargreavesi			
	A.* %	B.† %	Average %	2-hourly Total %	A.* %	B.† %	Average %	2-hourly Total %
16.15	0·1	0·1	0·1	0·3	0·1	0·0	0·1	0·4
17.15	0·1	0·1	0·1		0·0	0·1	0·1	
18.15	0·2	0·3	0·3	0·4	0·1	0·1	0·1	0·2
19.15	5·5	5·4	5·5		2·2	2·1	2·2	
20.15	6·5	7·1	6·8	12·3	3·1	3·0 .	3·0	5·2
21.15	7·8	7·7	7·8		5·4	4·7	5·0	
22.15	8·6	9·3	8·9	16·7	5·9	5·3	5·6	10·6
23.15	10·5	10·9	10·7		7·7	7·0	7·3	
00.15	9·6	9·4	9·5	20·2	10·1	9·7	9·9	17·2
01.15	9·7	9·3	9·5		8·7	9·4	9·1	
02.15	8·8	8·7	8·7	18·2	10·1	10·3	10·2	19·3
03.15	7·8	7·9	7·8		9·9	10·2	10·1	
04.15	8·2	7·6	7·9	15·7	10·4	10·9	10·7	20·8
05.15	7·1	6·9	7·0		9·2	9·5	9·4	
06.15	3·8	4·0	3·9	10·9	10·6	11·2	10·9	20·3
07.15	1·1	1·3	1·2		2·7	2·8	2·8	
08.15	1·0	0·9	0·9	2·1	1·3	1·3	1·3	4·1
09.15	1·0	1·0	1·0		0·8	0·8	0·8	
10.15	0·9	0·9	0·9	1·9	0·6	0·6	0·6	1·4
11.15	0·3	0·4	0·4		0·3	0·2	0·3	
12.15	0·4	0·3	0·4	0·8	0·2	0·2	0·2	0·5
13.15	0·2	0·2	0·2		0·1	0·1	0·1	
14.15	0·4	0·3	0·4	0·6	0·1	0·2	0·2	0·3
15.15	0·3	0·2	0·2		0·2	0·3	0·3	
Total	99·9	100·2	100·1	100·1	99·8	100·0	100·3	100·3

*A. Percentage of grand total.

†B. Average of percentages of individual catch totals.

Two methods of combining the results of individual catches are available. By the first method the figures for all the catches are added to give 24 separate totals, one for each hour. Each of these totals is then expressed as a percentage of the grand total of all the mosquitos of the species in question which were taken. The purpose of this reduction to percentages is to make possible a direct comparison between the figures for individual species irrespective of the total numbers taken. The method is of advantage in the case of species in which the smaller catches are so small as to be of doubtful significance since each catch is automatically weighted so that its contribution to the final figure is in proportion to its size. In the case of the two most abundant species, however, it has been thought advisable to employ in addition a second method. By this method the separate hourly figures for each individual

catch are expressed as percentages of the total taken in that particular catch. The 22 sets of percentages so obtained are then averaged to give a single percentage for each hour. By this means each catch is made to play an equal part in the final figure irrespective of its size. If it is possible to obtain a significant mean biting cycle for each species then, in theory, the two sets of figures obtained by these methods should approach one another more and more closely as the total numbers taken increase. In the case of the two species mentioned, *T. africanus* and *Anopheles hargreavesi*, this has proved to be so. Moreover, by comparing the two sets of figures, it is possible to form an idea of how many mosquitos of any given species would need to be taken to give a satisfactory mean biting cycle. The answer is seen to depend on the type of biting cycle involved. It is evident that in a species such as *T. africanus* in which the cycle is very " diffuse " and in which the time of the biting peak varies from one catch to another a much greater total number of mosquitos is required than in a species such as *Aëdes africanus* in which most of the biting takes place during a single hour.

The figures for *T. africanus* and *Anopheles hargreavesi* are given in Table VII. Those obtained by the first method are marked " A " and those obtained by the second method " B ". The curves shown in fig. 4 are based on the averages of the two sets of figures. These averages are also shown in Table VII. The curves are to be regarded merely as diagrams giving a simple picture of the mean biting cycles and, in order to smooth and simplify them, they are based on 2-hourly totals. These totals represent the numbers of mosquitos collected at the end of 12 consecutive 2-hour periods and can be arrived at in two different ways depending on whether the mosquitos are assumed to have been collected at the odd or the even hours.
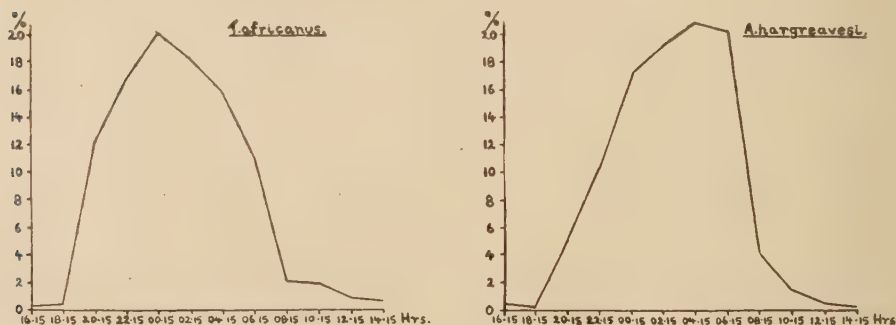
Fig. 4.—Biting cycles of *T. africanus* and *A. hargreavesi* at Itowolo.

Since the majority of species exhibit a well-marked biting peak occupying a single hour it is necessary in each case to select the method of grouping which gives the most faithful picture of the hour to hour cycle. Thus, for example, some species show very high biting activity between 18.15 and 19.15 hrs. following on little or no biting between 17.15 and 18.15 hrs. but with a fairly high level between 19.15 and 20.15 hrs. Such a biting peak is very significant but in a 2-hourly curve based on the odd hours it would be diminished or even obliterated since the high figure for 19.15 hrs. would be combined with a very low one for 18.15 hrs. In such a case it is obviously better to combine the figure for 19.15 hrs. with that for 20.15 hrs., *i.e.*, to base the curve on the even hours. In fig. 4 the curve for *T. africanus* is based on the even hours in order to show the sharp changes in biting activity associated with nightfall and daybreak. In the case of *Anopheles hargreavesi* the same method has been adopted since only in this way is it possible to give full value to the high biting

TABLE VIII.

Biting Cycles of *A. gambiae* and *A. africanus* in Bwamba and at Itowolo.

	Bwamba					Itowolo			
Time (L.M.T.)	*A. gambiae* %	%	*A. africanus* %	%	Time (L.M.T.)	*A. gambiae* %	%	*A. africanus* %	%
16.00	0·3	0·4	0·9	2·0	16.15	0·1		3·2	4·2
17.00	0·5		0·6		17.15	0·0	0·1	3·4	
18.00	0·8	1·3	3·9	4·5	18.15	0·0		6·3	9·7
19.00	3·3		42·1		19.15	5·2	5·2	21·3	
20.00	3·6	6·9	16·4	58·5	20.15	2·0		7·2	28·5
21.00	4·2		6·7		21.15	2·7	4·7	3·8	
22.00	5·5	9·7	3·5	10·2	22.15	4·5		5·5	9·3
23.00	6·2		3·5		23.15	6·1	10·6	5·7	
00.00	6·5	12·7	2·6	6·1	00.15	7·2		5·0	10·7
01.00	7·0		1·1		01.15	8·1	15·3	4·4	
02.00	7·7	14·7	1·3	2·4	02.15	9·7		2·9	7·3
03.00	9·3		1·7		03.15	10·1	19·8	2·7	
04.00	9·9	19·2	1·1	2·8	04.15	14·8		6·3	9·0
05.00	12·7		0·4		05.15	16·8	31·6	1·9	
06.00	14·4	27·1	2·2	2·6	06.15	7·4		5·7	7·6
07.00	4·9		2·8		07.15	1·8	9·2	4·2	
08.00	1·2	6·1	2·2	5·0	08.15	0·8		1·3	5·5
09.00	0·7		1·3		09.15	0·2	1·0	2·1	
10.00	0·4	1·1	0·9	2·2	10.15	0·8		0·6	2·7
11.00	0·3		1·5		11.15	0·2	1·0	1·7	
12.00	0·3	0·6	0·4	1·9	12.15	0·8		0·8	2·5
13.00	0·1		0·6		13.15	0·5	1·3	1·0	
14.00	0·2	0·3	1·3	1·9	14.15	0·2		2·1	3·1
15.00	0·1		1·1		15.15	0·2	0·4	1·0	
Total	100·1	100·1	100·1	100·1	Total	100·2	100·2	100·1	100·1

activity during the hour ending 06.15. The figures for *A. gambiae* and *Aëdes africanus* given in Table VIII were obtained by method " A ", *i.e.*, by simple addition of the totals for each hour and subsequent conversion into percentages of the grand total since the numbers taken in the smaller catches were too small for method " B " to be applicable. The 2-hourly curve for *Anopheles gambiae* is shown in fig. 5 together with one based on figures obtained by a similar technique in Bwamba County, Uganda (Haddow, 1947). The 15 minutes overlap in the collecting times has rendered it necessary to base the Bwamba curve on the even hours and the Itowolo curve on the odd hours in order to give full value to the morning peak in both cases.

Anopheles gambiae.

This species is discussed first because very large figures from Uganda are available for comparison. In all more than 30,000 *gambiae* (30,240) were taken in Bwamba County (Haddow, 1947) and, in view of the relatively small number (2,502) taken at Itowolo, the agreement between the two sets of data is felt to be very satisfactory (fig. 5). The Bwamba and Itowolo figures are shown together for comparison in Table VIII and it will be seen that, whereas the peak biting time in Bwamba falls between 05.00 and 06.00 hrs., that at Itowolo falls between 04.15 and 05.15 hrs. It is a not unreasonable hypothesis that, on an average, the absolute peak is reached in both localities between 05.00 and 05.15 hrs., *i.e.*, during the period of Nautical Twilight (sun 6°–12° below the horizon). At the evening end of the cycle a sharp initial rise in biting activity takes place in Bwamba between 18.00 and 19.00 hrs. and at Itowolo between 18.15 and 19.15 hrs. An examination of the figures leads to the conclusion that this rise takes places in both localities between 18.15 and 19.00 hrs.

The sharper rise at Itowolo may then be due in part to the higher biting rate between 19.00 and 19.15 than between 18.00 and 18.15 hrs. The small peak which occurs at Itowolo at this time and which shows clearly in the figures, although it is almost obliterated in the curve by the method of 2-hourly grouping, is thought to be significant since it appeared in most of the catches in which *gambiae* was abundant. If the high biting activity responsible for this peak was confined to the period 18.45–19.15 hrs. it would show in the Itowolo figures but in Bwamba it would be shared by the totals for 19.00 and 20.00 hrs. That something of this sort happens is suggested by the fact that, in the latter locality, the totals for these two hours are nearly equal although they follow on a sharp rise in biting activity. In consequence the hourly curve for Bwamba is flattened at this point and shows a marked inflection corresponding to the small peak in the Itowolo curve. Summarising the results from both localities it may be said that the mean biting curve for *A. gambiae* shows an initial rise during the hour after sunset, probably accompanied by a small peak during the period of Nautical Twilight, rises steadily during the night, reaching its maximum during the hour before sunrise, probably again during the period of Nautical Twilight, and falls away rapidly thereafter to a minimal level which is maintained throughout the day. It is to be hoped that it will be possible to check the precise times of the morning and evening peaks in the field by making collections at short intervals during the critical periods. The small amount of indirect evidence at present available (Ribbands, 1946, Thomson, 1948) does not suggest that any great difference is to be found between the biting cycles of *A. gambiae* and *A. melas*. Had a marked difference existed it might have been expected to lead to a greater discrepancy between the figures from Itowolo and those from Bwamba where the latter species is unlikely to be represented.
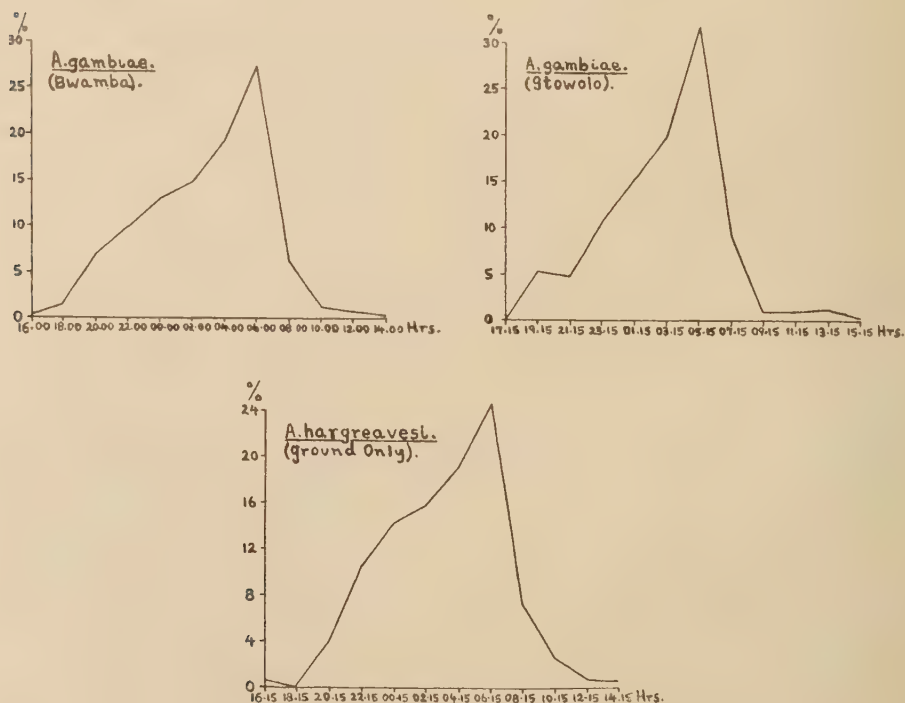
Fig. 5.—Biting cycles of *A. gambiae* at Bwamba and Itowolo and of *A. hargreavesi* (ground only) at Itowolo.

Aëdes africanus.

It is particularly fortunate that both Bwamba and Itowolo yielded good numbers of this species since it is of special interest in connection with jungle yellow fever. Its biting curve stands in sharp contrast to that of *A. gambiae* since the main peak is associated with evening instead of morning twilight. Two-hourly curves from both localities are shown in fig. 6. They are based on the even hours in order to do justice to the evening peak. The figures on which they are based are given in Table VIII. The figures from Bwamba are based on a total of 463 mosquitos, those from Itowolo on a total of 525. Comparing the two sets of data it will be seen that in Bwamba the evening peak occurs between 18.00 and 19.00 hrs. whereas at Itowolo it occurs between 18.15 and 19.15 hrs. The inference is that in both localities maximum biting activity is reached between 18.15 and 19.00 hrs. Kerr (1933) gives combined half-hourly figures for this species and the closely related *A. luteocephalus*, Newst., in the
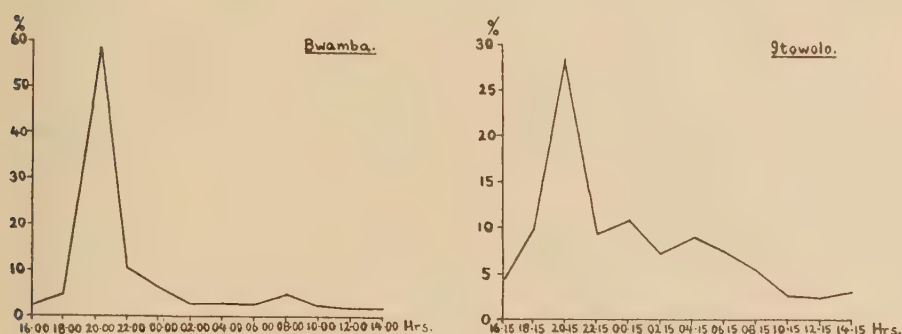
Fig. 6.—Biting cycles of *A. africanus* in Bwamba and at Itowolo.

neighbourhood of Lagos. Studies made by the present author at Ogbomosho in southwestern Nigeria suggest that these two species have closely similar biting cycles and, since *A. africanus* made up over two-thirds of Kerr's total catch, his figures may be taken as representative of the behaviour of this species. He does not state which time scale he employed but from internal evidence it is clear that it was Nigerian Standard Time. Reducing his times to L.M.T. it is found that he obtained, in all, 20 mosquitos between 18.15 and 18.45 hrs. and 9 between 18.45 and 19.15 hrs. This is in good agreement with the inference drawn from the Bwamba and Itowolo figures and it appears reasonable to conclude that the mean biting curve of *A. africanus* reaches its absolute peak between 18.15 and 18.45 hrs. in all three localities, *i.e.*, at about the same time as the small evening peak postulated for *Anopheles gambiae* or a little earlier during the period of Civil Twilight. It is again to be hoped that the times will be defined more precisely by catches made at short intervals in the field during the critical period. The difference between the Bwamba and Itowolo curves at the evening end of the cycle appears to be mainly a quantitative one, the Bwamba peak being almost twice as high as that at Itowolo. The discrepancy can be accounted for in part by the staggering of the time scales but this explanation is not wholly adequate and there remains a real discrepancy which cannot for the moment be explained. Both the Bwamba and Itowolo curves show a small morning peak covering a period from some time during Nautical Twilight until well after sunrise. At Itowolo, where it is more pronounced, the peak is a very complex one and is accompanied and probably conditioned by changes in vertical distribution. It is discussed in detail below in connection with vertical distribution cycles.

Summarising the mean biting cycle for *A. africanus* it may be said that a sharp rise takes place shortly after sunset leading to a very pronounced peak during the period of Civil or Nautical Twilight. After this biting falls away and is maintained at a low level during the night. The small morning peak is of a complex nature and is associated with changes in vertical distribution. Biting continues at a low level throughout the day.

Anopheles hargreavesi.

This was the second most abundant species at Itowolo. It is a known malaria carrier (Barber & Olinger, 1931) and in view of its larger numbers and more uniform seasonal distribution may well be a more important vector in this area than *A. gambiae*. The mean biting curves of the two species appear at first sight to be very different (figs. 4 and 5) but on closer examination the difference is seen mainly to affect the upper platforms. This is shown clearly by the curve for *A. hargreavesi* for the ground only which closely resembles that for *A. gambiae* (fig. 5). The main biting peak occurs an hour later than in *gambiae* and is accordingly conditioned by quite a different vertical distribution. It is discussed in detail below under vertical distribution cycles.

Summarising the mean biting curve for this species it may be said to show the same sharp initial rise associated with evening twilight as was observed in *A. gambiae* but without any apparent evening peak. Instead it continues to rise rapidly, reaching a high level by midnight at which it remains until sunrise after which it falls steeply to the daytime level.

Taeniorhynchus africanus.

The mean biting curve for this species shows conspicuous differences from those previously discussed (fig. 4). It shows no morning or evening peak and the main peak, which occurs in the middle of the night, varied from catch to catch. On the average it occurred during the hour ending 23.15 but in individual catches it might be found at any time from 21.15 to 05.15 hrs. Haddow (1945, 1947) gives 4-hourly figures for this species, which was not abundant in Bwamba (Table IX). He found maximum biting during the period ending 22.00 hrs. At Ogbomosho during two 24-hour catches carried out by the present author the peaks occurred at 23.15 and 00.15 hrs., respectively. It is therefore necessary in the present case to explain a biting curve with a peak varying in the time of its occurrence but always to be found at some time during the middle part of the night. Haddow considers that changes in temperature and humidity may be involved and the possible effects of moonlight cannot be overlooked but in the opinion of the present author this curve like all the others obtained at Itowolo can be explained in terms of the changes in activity associated with sunset and daybreak. If the curve for *A. hargreavesi* (fig. 4 and

TABLE IX.

Four-hourly Biting Activity of *Taeniorhynchus africanus* in Bwamba and at Itowolo.

Bwamba		Itowolo	
4 Hours ending*	% of Total Catch	4 Hours ending*	% of Total Catch
14.00	3·6	14.15	1·4
18.00	2·8	18.15	0·7
22.00	48·0	22.15	29·0
02.00	18·1	02.15	38·4
06.00	20·6	06.15	26·6
10.00	6·9	10.15	4·0
Total	100·0		100·1

*L.M.T. for both localities.

Table VII) is combined with its mirror image the resultant curve agrees very closely with that obtained for *T. africanus*, differing mainly in being symmetrical. In other words the *T. africanus* curve could be produced by two sections of the population, one showing maximum biting activity in the evening, the other in the morning. The asymmetry of the curve would, of course, be due to a disparity in the numbers showing the two types of behaviour and changes in the proportions of these two sections of the population from catch to catch would account very satisfactorily for the variations in the time of occurrence of maximum activity. If this explanation were to hold good it would be necessary for the two sections of the population to have convex curves similar to that shown by *A. hargreavesi*. Concave curves with a marked peak like that shown by *A. africanus* would produce a bi-modal resultant curve with sharp morning and evening peaks. It is perhaps of interest to note that a curve of precisely this kind was found in the case of *Aëdes irritans*, Theo., which is to be discussed in the second paper of the present series. The concept of two sections of the population with differing behaviour is a very attractive one since it accounts with singular aptness for all the peculiar features of the *T. africanus* curve. It would also explain the very curious vertical distribution recorded for this species from Uganda (Haddow, 1947), which is discussed below.

Summarising the mean biting curve of this species it may be said to show the same sharp rise in biting activity during the period of evening twilight as was observed in other species. After this the biting rate continues to increase reaching its maximum at some time during the middle part of the night, the exact hour varying from catch to catch. It then falls away gradually until daybreak when it falls rapidly to the daytime level. In the author's opinion the occurrence of the biting peak is due to the presence of two sections of the population in such numbers that their combined total is greater at the peak hour than at any other time during the night although their individual biting peaks are associated with the periods of morning and evening twilight respectively.

Vertical Distribution.

To facilitate direct comparison, the total numbers of mosquitos occurring on each platform have been reduced to percentages of the grand totals. These percentages are given in Table X.

TABLE X.

Vertical Distribution at Itowolo.

Platform	Height	*A. gambiae* %	*A. africanus* %	*A. hargreavesi* %	*T. africanus* %
IV	52 feet	4·2	42·7	3·2	5·3
III	40 ,,	11·8	27·2	10·6	14·9
II	22 ,,	34·7	22·7	36·7	25·4
I	0 ,,	49·3	7·4	49·5	54·5
	Total	100·0	100·0	100·0	100·1

Anopheles gambiae.

The very close correspondence between the figures for this species and those for *A. hargreavesi* is interesting. Haddow (1947) gives figures for two localities in Bwamba County which are shown, reduced to percentages, in Table XI. It will be seen that there are considerable differences between the three localities, those in Bwamba showing a greater proportion on the ground, although when the two bottom platforms are taken together the agreement between Mongiro and Itowolo is exact. It would seem that vertical distribution in this species is very much affected by local conditions.

At Itowolo the proportions varied considerably from catch to catch and from hour to hour during the same catch. In a few of the smaller catches the total taken on the second platform exceeded that taken on the ground.

TABLE XI.

Vertical Distribution in Bwamba.

Plat-form	Mongiro				Mamirimiri			
	Height ft.	*A. gambiae* %	*A. africanus* %	*T. africanus* %	Height ft.	*A. gambiae* %	*A. africanus* %	*T. africanus* %
V	—	—	—	—	82	2·2	20·2	21·3
IV	54	5·2	53·1	30·7	58	2·5	46·3	21·3
III	31	10·8	27·1	20·0	44	3·2	21·0	8·5
II	16	17·7	15·6	14·7	22	10·3	7·6	10·6
I	0	66·3	4·2	34·7	0	81·7	4·9	38·3
	Total	100·0	100·0	100·1		99·9	100·0	100·0

Aëdes africanus.

It is interesting to note that at Mongiro the concentration of this species at the top of the tree was greater than at Itowolo while at Mamirimiri it was greater still. Similar differences were shown by the concentrations of *A. gambiae* on the ground and it is possible that they reflect differences of gradient in the physical factors operating in the three localities.

Taeniorhynchus africanus.

The figures from Mongiro and Mamirimiri are small but they agree in showing more mosquitos on the top than on the middle platforms. This is in marked contradistinction to the state of affairs at Itowolo where the steady decline in numbers from the ground to the top platform was constant in all catches. In the author's opinion this type of distribution may well be explained by a difference in activity between two sections of the population similar to that already invoked to explain the pecularities of the biting cycle.

Vertical Distribution Cycles.

In all the species under consideration biting activity was much reduced during the daytime. Moreover this reduction was accompanied in all cases by a depression in vertical distribution. Thus it will be seen from Table I that in all the species the proportion of mosquitos biting on the upper platforms to those biting on the ground was steadily reduced from daybreak onwards until the daytime level was reached. During most of the period of full daylight biting was confined almost entirely to the ground or at most to the two bottom platforms. Only in *Aëdes africanus*, however, was there any evidence of an actual movement of mosquitos from the upper levels to the ground. In this case the occurrence of such a movement is suggested by the fact that biting on the ground was very much heavier during the daytime than at night whereas at the top of the tree the reverse was true. Thus on platform IV, 214 mosquitos of this species were taken between 18.15 and 06.15 hrs. and 10 between 06.15 and 18.15 hrs. while on the ground the corresponding figures were 5 and 24, respectively. That a similar descent may take place during the peak biting period if the bait is concentrated on the ground is suggested by the figures given by Kerr (1933). During 10 all-night catches, made on the ground only, he took 108 *Aëdes africanus*. During the same period of the night 22 catches at Itowolo yielded only 6 mosquitos of this species. In view of the enormous population of *A. africanus* which, in Kerr's case, a normal vertical distribution would imply and the fact that he was

working only a few miles from Itowolo it seems improbable that the difference can be explained merely by the presence of more abundant breeding places. The figures are, however, too small to warrant a definite conclusion and our knowledge of variation in behaviour from one locality to another is very slight. The point is an important one in considering the part played by *Aëdes africanus* in the epidemiology of jungle yellow fever. It could be checked by quite simple experiments in the field.

In the case of *Anopheles hargreavesi*, as has already been stated, the depression in vertical distribution associated with daybreak is so pronounced as markedly to affect the shape of the mean biting curve. In this species the biting curve for the ground platform resembles that of *A. gambiae* (fig. 5) although the curve based on all four platforms taken together differs considerably (fig. 4). This is because the main biting peak in this species occurs later than in *gambiae* and is delayed until after sunrise. In consequence it occurs at a time when biting on the upper platforms has been much reduced. Accordingly the latter do not contribute to the peak figure to the same extent that they do in *gambiae* and the peak as a whole is lowered (Table I).

At the evening end of the cycle all the three principal ground biting species showed heavier concentrations on the upper platforms, the peak hours in this respect being 18.15 to 20.15 in the case of *T. africanus*, 19.15 to 21.15 in the case of *Anopheles hargreavesi* and 20.15 to 22.15 in the case of *A. gambiae*. The changes involved were complex and can best be understood from the actual figures which are given in Table I. The vertical distribution of males of *T. africanus* suggests that the concentration of females on the upper platforms may be associated with swarming. This question will be discussed in more detail later in a second paper.

With regard to long-term changes in vertical distribution Haddow (1947) noted a tendency for certain species to be more abundant at the preferred level during the dry than during the wet season. At Itowolo only *T. africanus* showed such a tendency and in the other three species under consideration it was reversed. In all cases, however, the difference was slight and it is clear that investigations would have to be carried out over a long period before definite conclusions could be reached.

Summary.

The present paper is the first of two dealing with the seasonal distribution, hourly variation in biting activity, and vertical distribution of certain Southern Nigerian mosquitos. The technique employed in the studies under discussion resembled that used by Haddow (1947) in Uganda. Human bait was used in both cases and the agreement between the results obtained in the two localities is felt to be such as fully to justify the employment of such methods. Over fifty species of mosquitos were taken in all and fourteen of these proved to be sufficiently abundant to yield significant figures. Four of these are discussed and the remainder will be dealt with in the second paper.

The most remarkable feature of the seasonal distribution was the very restricted period during which *Anopheles gambiae* was present in any abundance. This species occurred in large numbers only during the heavy rains of June and July and, to a less extent, in association with the small rains of September -October. During the dry season it almost disappeared. *Aëdes africanus* showed a similar distribution to that of *gambiae* but *Taeniorhynchus africanus* and *Anopheles hargreavesi* were most numerous during the dry season. It is suggested that in the area under consideration the latter species may well be a more important malaria vector than *gambiae* owing to its greater abundance and more uniform seasonal distribution.

As in Uganda *Aëdes africanus* proved to be mainly a tree-top biter with a well-marked peak period of biting activity associated with evening twilight while *A. gambiae* and *A. hargreavesi* bit mainly on the ground and had their peak period in the morning. The biting cycle (hourly variation in biting activity) of *hargreavesi*

showed considerable differences from that of *gambiae* but on analysis these proved
to be superficial and to be due mainly to a difference of about one hour in the times
of occurrence of their peak biting periods and a corresponding change in the vertical
distributions with which these were associated. The occurrence of peak activity
in the early morning in *A. gambiae* is considered to have an important bearing on
the question of personal precautions against malaria.

The biting cycle of *T. africanus* differed from that of any other species studied in
that the period of peak activity occurred during the middle part of the night, the
exact time varying quite widely on the various occasions on which observations
were made. It is suggested that this may have been due to the occurrence of both
morning and evening peak periods within the same species.

Details are given of the variations in temperature and humidity encountered
during the experiments together with meteorological observations made at a neigh-
bouring airfield but a full discussion of the relation of these factors to the activity
of the mosquitos is reserved for the second paper when all the data will have been
presented.

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THE MOSQUITOES OF BWAMBA COUNTY, UGANDA.*

VII.—INTENSIVE CATCHING ON TREE-PLATFORMS, WITH FURTHER OBSERVATIONS ON *AËDES (STEGOMYIA) AFRICANUS*, THEOBALD.

By A. J. Haddow and A. F. Mahaffy.

Yellow Fever Research Institute, Entebbe, Uganda.

The isolation of yellow fever virus from mosquitoes caught in uninhabited rain-forest in Bwamba County, Uganda, has been described elsewhere (Smithburn & Haddow, 1946). The virus was isolated in 1944 from a mixed lot of *Aëdes* spp. taken in swamp-forest at Mongiro in the northern part of the Semliki Forest, which covers much of the Bwamba lowlands. The discovery of virus was followed by intensive work at Mongiro and in the neighbouring forest area of Mamirimiri. A series of continuous 24-hour mosquito catches were made simultaneously at ground-level and at various heights in the trees of both areas. Yellow fever virus was not again isolated, but much entomological information was gained. The results of this work, which was carried out in 1944 and early 1945, have been published in a previous paper (Haddow & others, 1947a) to which reference should also be made for a description of the swamp-forest at Mongiro and Mamirimiri. For a more general description of Bwamba County, a heavily-forested area in western Uganda where yellow fever is endemic, the first paper of the present series (Haddow, 1945) should be consulted.

As a result of the 24-hour catches mentioned above and of observations on the incidence of immunity to yellow fever among wild monkeys in Bwamba (Haddow & others, 1947b), it had been concluded that the mosquito most likely to be involved in the transmission of yellow fever among the monkeys of that area was *Aëdes (Stegomyia) africanus*, Theo. This species has a wide distribution in the central African forests. It survives the dry season in the adult state in forested and wooded areas in Uganda, is an efficient vector of yellow fever in the laboratory, and was one of the 12 species included in the infected material taken from swamp-forest at Mongiro in 1944. More particularly, it shows a well-marked peak of biting activity just after sunset in the forest canopy, which is its preferred habitat. This is a point of importance, as monkeys normally repair before sunset to trees in which they pass the night, and are asleep by the time that *A. africanus* reaches its highest activity.

In view of these facts, we decided to make large-scale catches of *A. africanus* in trees with the hope of isolating virus from the captured mosquitoes by allowing them to bite a non-immune rhesus monkey in the laboratory and by triturating them and inoculating them into laboratory animals. Since the available evidence had led to the conclusion that yellow fever is endemic among monkeys throughout the Bwamba lowlands, it seemed that there was as good a chance of isolating the virus by continued work at Mongiro and Mamirimiri as there would be if the work was shifted to another locality. We therefore carried out an intensive series of catches in trees in these areas during part of 1945, inoculating the material obtained into rhesus

A

monkeys in the Bwamba field laboratory.   Yellow fever virus was not obtained during
this investigation, but further knowledge of the arboreal mosquitoes was gained, and
methods of inoculating laboratory animals in the field were thoroughly tested.   The
purpose of the present paper is to discuss these studies briefly.

Catching-methods.

Since we wished to collect as many *A. africanus* as possible, we planned to make
a long series of catches in the forest canopy during the sunset period.   The experi-
mental platforms used at Mamirimiri during the investigation of 1944–45 (22, 44,
58 and 82 feet above ground) and at Mongiro (16, 31 and 54 feet above ground) were
still in good condition, and we decided to use them for this purpose.   Almost immedi-
ately after they had been brought into use, it was found that the 16- and 31-foot
platforms at Mongiro were yielding a very small number of *A. africanus*.   These
were therefore dismantled, and a new platform 51 feet high was built in their place.
In addition, 4 new platforms (55, 56, 58 and 59 feet above ground) were built at
Mongiro.   Two boys acting as bait worked on each platform 5 days a week.   Later,
in order to increase the yield, 3 boys were placed on each platform, and they worked
6 days a week.   Catching was continued for a total of 11 weeks, covering the period
from April to June 1945.   Catches were always begun in the late afternoon (16.00–
17.00 hours, local mean time) and were continued till well after dark (20.30–21.00
hours, local mean time).   All mosquitoes were caught individually in short test-
tubes and the tubes then plugged lightly with cotton wool.

During the first 2 weeks' work at Mongiro, a ground-level control unit was used
in connection with each of the 5 high platforms then available.   The object of this
procedure was to confirm previous findings on the vertical distribution of mosquitoes
in the area.

In addition to mosquitoes, large numbers of an arboreal Tabanid, *Chrysops
centurionis*, Aust., were included in the catches.   Observations on this species and
on other arboreal TABANIDAE collected during 1944 and 1945 will form the subject
of a separate communication.

Catches on low Platforms at Mongiro.

Two catches, totalling 12 man-hours, were made on the 16-foot platform and 7,
totalling 46 man-hours, on the 31-foot platform.   The total represented 58 man-
hours.   The mosquitoes taken were :—

Anopheles (*Myzomyia*) *gambiae*, Giles ...	...	...	46
Uranotaenia ornata var. *musarum*, Edw.	...	...	1
Aëdes (*Stegomyia*) *apicoargenteus*, Theo.	...	...	1
A. (*S.*) *africanus*, Theo.	...	...	3
A. (*Aëdimorphus*) *natronius*, Edw. ...	...	...	1
Culex spp. indet.	...	...	29
			—
Total ...	...	...	81
			—

As mentioned above, these stations were then dismantled.   The results call for
no special comment, apart from mention of the occurrence of *U. ornata* var. *musarum*.
This species is not known to bite man in Bwamba but, together with some other
species of *Harpagomyia*, *Uranotaenia*, *Ficalbia* and *Megarhinus*, it sometimes alights
on the catchers, apparently to suck sweat from the skin.

Catches at Mamirimiri.

At Mamirimiri 58 catches, totalling 2,251 man-hours, were made. On account of the pressure of work, mosquitoes from all levels were combined in one lot in the field. The sample is thus a very generalised one, including material taken at levels ranging from 22 to 82 feet above ground, all obtained in trees during the evening and early night. The results of these catches were as follows :—

Anopheles (Myzomyia) funestus, Giles	4
A. (M.) gambiae, Giles	600
Harpagomyia taeniarostris, Theo.	1
Uranotaenia ornata var. *musarum*, Edw.	2
Taeniorhynchus (Coquillettidia) pseudoconopas, Theo....	1
T. (C.) fuscopennatus, Theo. ... ... —... ...	26
T. (C.) aurites, Theo.	2
T. (C.) aureus, Edw.	3
T. (C.) microannulatus, Theo.	1
T. (Mansonioides) africanus, Theo.	517
T. (M.) uniformis, Theo.	3
Aëdes (Mucidus) grahami, Theo.	22
A. (Finlaya) longipalpis, Grünb.	1
A. (F.) ingrami, Edw.	47
A. (Stegomyia) aegypti, L.	2
A. (S.) apicoargenteus, Theo.	43
A. (S.) africanus, Theo. 1,463
A. (Aëdimorphus) tarsalis, Newst. group	4
A. (A.) natronius, Edw.	42
Culex spp. indet.	278
Total 3,062

These figures show a very marked preponderance of *A. africanus* among the arboreal mosquitoes in the sunset period. In the sample it forms 48 per cent. of the total catch, or 60 per cent. of the total Culicines. It must be understood that this observation applies only to the evening and early night, for it has been shown (Haddow & others, 1947a) that at Mongiro and Mamirimiri *A. longipalpis* and *A. apicoargenteus* predominate by day in the canopy, while during the later hours of the night *A. gambiae* is the commonest biting species at all levels.

A point of special interest is the occurrence of *A. aegypti* in trees, in uninhabited rain-forest. The unusual habits of this important species in Bwamba have been discussed previously (Haddow, 1945), but this is the first record of its presence in Bwamba above ground-level. The finding is not unprecedented, as *A. aegypti* has been taken at heights up to about 30 feet in the Langata Forest, Kenya, by Mrs. Ellinor C. van Someren (private communication).

Finally, the occurrence of *Taeniorhynchus* spp., and more particularly the prevalence of *T. africanus* should be noted. It must be remembered that this species is known to be capable of transmitting yellow fever virus under laboratory conditions (Philip, 1930), that it has a very wide distribution and a wide flight-range, and that as well as biting freely in many kinds of open and forested country, it is quite abundant in huts, houses and tents.

Catches on high Platforms at Mongiro.

At Mongiro 64 catches, totalling 3,158 man-hours, were made on the high plat-forms. As all the platforms concerned were between 50 and 60 feet above ground, this sample of the arboreal mosquitoes active during the evening and early night is a fairly critical one. The results were as follows :—

Anopheles (*Myzomyia*) *funestus*, Giles	6
A. (*M.*) *gambiae*, Giles	698
Taeniorhynchus (*Coquillettidia*) *maculipennis*, Theo. ...	1
T. (*C.*) *fuscopennatus*, Theo.	8
T. (*Mansonioides*) *africanus*, Theo.	302
T. (*M.*) *uniformis*, Theo.	1
Aëdes (*Mucidus*) *grahami*, Theo.	69
A. (*Finlaya*) *longipalpis*, Grünb.	3
A. (*F.*) *ingrami*, Edw.	58
A. (*Stegomyia*) *aegypti*, L.	13
A. (*S.*) *apicoargenteus*, Theo.	163
A. (*S.*) *de-boeri* subsp. *de-meilloni*, Edw.	1
A. (*S.*) *africanus*, Theo.	2,113
A. (*Aëdimorphus*) *haworthi*, Edw.	1
A. (*A.*) *tarsalis*, Newst. group	33
A. (*A.*) *lamborni*, Edw.	1
A. (*A.*) *cumminsi*, Theo.	2
A. (*A.*) *natronius*, Edw.	54
Eretmapodites chrysogaster, Graham group	3
E. inornatus, Newst. group	1
Culex spp. indet.	245
Total	3,776

As in the catches from Mamirimiri, the preponderance of *A. africanus* in the canopy during the sunset period is very clearly shown. In this sample it forms 56 per cent. of the total catch, or 69 per cent. of the total Culicines.

The catch of 13 *A. aegypti* actually in the canopy is of outstanding interest. Whereas the Mamirimiri catches covered a wide vertical zone, the Mongiro series was confined to the thickest part of the main canopy. At the time when this work was carried out, native huts were exceedingly scarce in the Mongiro-Mamirimiri area and the nearest was a full half mile from the catching-stations.

Once more the prevalence of *T. africanus* in the forest canopy is to be noted as a point of possible importance. Its regular occurrence in this habitat has been amply confirmed by subsequent work still in progress.

In the Mongiro series of catches, the percentages of such ground-haunting mosquitoes as the *Eretmapodites* and *Aëdimorphus* spp. taken is much higher than was usual in previous and subsequent catches. The reason for this is not under-stood, but it may be mentioned that the great majority caught in trees were taken on a single night. This seems to imply that some microclimatic incident (imper-ceptible to the observers) may have been responsible.

This note is not intended to cover the behaviour of *A. natronius* which (alone of the local *Aëdimorphus* spp.) seems always to exhibit fairly well-marked arboreal tendencies.

Catches at Mongiro with Ground-level Controls.

During the first 2 weeks' work at Mongiro, mentioned above, ground-level control units were stationed below each of the 5 high platforms then in use. Thus at each station 2 catchers worked on the platform and 2 sat below the tree. The catches were made simultaneously with the object of confirming previous findings in regard to vertical distribution of mosquitoes in forest. Ten such catches were made, totalling 300 man-hours in the canopy and 300 at ground-level. The mosquitoes taken on the platforms are included in the Mongiro totals given above. It seems worth while to give the results separately because the series so clearly shows the difference between the arboreal and ground-level mosquito populations in the sunset period.

	Platforms	Ground-level
Anopheles (*Myzomyia*) *funestus*, Giles	1	—
A. (*M.*) *gambiae*, Giles	109	796
Taeniorhynchus (*Coquillettidia*) *fuscopennatus*, Theo. ...	—	2
T. (*Mansonioides*) *africanus*, Theo.	3	—
Aëdes (*Mucidus*) *grahami*, Theo.	1	—
A. (*Finlaya*) *ingrami*, Edw.	1	5
A. (*Stegomyia*) *aegypti*, L.	1	2
A. (*S.*) *apicoargenteus*, Theo.	4	1
A. (*S.*) *de-boeri* subsp. *de-meilloni*, Edw.	—	1
A. (*S.*) *africanus*, Theo.	123	14
A. (*Aëdimorphus*) *argenteopunctatus*, Theo.	—	1
A. (*A.*) *tarsalis*, Newst. group	3	24
A. (*A.*) *lamborni*, Edw.	—	2
A. (*A.*) *natronius*, Edw.	4	8
A. (*Banksinella*) *circumluteolus*, Theo.	—	3
A. (*Dunnius*) *kummi*, Edw.	—	1
Eretmapodites chrysogaster, Graham group	—	2
E. inornatus, Newst. group	—	1
Culex (*Lutzia*) *tigripes*, Grp. & C.	—	1
Culex spp. indet.	51	270

The outstanding point is, of course, the prevalence of *A. africanus* on the platforms, where it formed 41 per cent. of the total catch or 64 per cent. of the total Culicines taken, whereas at ground-level it formed only 1 per cent. of the total catch or 4 per cent. of the total Culicines.

The mosquitoes taken at ground-level during this series were inoculated into laboratory animals along with the other material.

The Effect of general Weather Conditions on the Biting-activity of *A. africanus*.

It has previously been pointed out (Haddow & others, 1947a) that *A. africanus* is peculiarly susceptible to the influence of weather conditions. On warm evenings after a sunny afternoon it bites freely. Anything which causes a marked fall in temperature at sunset, such as wind, rain, or even heavy clouds, greatly reduces its activity. On such unfavourable evenings the post-sunset peak of biting-activity may be very slight or even absent. It is usual on these occasions to find larger numbers biting during the later night hours than is normally observed. But even then the totals seldom if ever reach the yield to be expected on fair evenings.

During the present work *A. africanus* was the only mosquito taken on every night of the series, but the numbers fluctuated very greatly from day to day. The poorest catch was 5, and the best 236. In almost every case the occurrence of a poor catch could at once be correlated with chilly conditions. The figures for the last 6 weeks' work at Mongiro may be quoted to demonstrate this point. During this

period there were 6 high platforms in use and 6 catches a week were made. The sample therefore is a fairly uniform one. Since the total man-hours varied slightly from week to week, it is necessary to standardise the crude figures, by taking the catching rate rather than the total per night. If the catch per 10 man-hours be taken as the unit, the following figures are obtained :—

Date				Catch per 10 man-hours	Date				Catch per 10 man-hours
14 May	...	...	...	1**	4 June	...	...	...	8
15 ,,	...	...	...	3**	5 ,,	...	...	...	6
16 ,,	...	...	...	10	6 ,,	...	...	...	10
17 ,,	...	...	...	14	7 ,,	...	...	...	22
18 ,,	...	...	...	8	8 ,,	...	...	...	9
19 ,,	...	...	...	2**	9 ,,	...	...	...	23
20 ,,	...	...	...	No catch	10 ,,	...	...	...	No catch
21 ,,	...	...	...	11	11 ,,	...	...	...	11
22 ,,	...	...	...	3**	12 ,,	...	...	...	15
23 ,,	...	...	...	4**	13 ,,	...	...	...	14
24 ,,	...	...	...	4**	14 ,,	...	...	...	2**
25 ,,	...	...	...	6	15 ,,	...	...	...	9
26 ,,	...	...	...	2**	16 ,,	...	...	...	5
27 ,,	...	...	...	No catch	17 ,,	...	...	...	No catch
28 ,,	...	...	...	2**	18 ,,	...	...	...	4
29 ,,	...	...	...	11	19 ,,	...	...	...	2**
30 ,,	...	...	...	11	20 ,,	...	...	...	4**
31 ,,	...	...	...	8	21 ,,	...	...	...	8
1 June	...	...	...	18	22 ,,	...	...	...	10
2 ,,	...	...	...	13	23 ,,	...	...	...	34
3 ,,	...	...	...	No catch	24 ,,	...	...	...	No catch

It will be seen that, while the catching-rate varied very greatly from night to night, it fell to less than 5 per 10 man-hours on 12 occasions only. On 11 of these, marked with asterisks, the poor yield could at once be correlated with cold weather conditions.

Anopheles gambiae reaches its main biting-activity in the hours before dawn and the principal difference between this period and that at which *A. africanus* is most active is one of temperature. The post-sunset period is relatively warm and that before dawn is much colder. The other main factor is that the saturation deficiency remains relatively constant and always very low during the night hours in the Semliki Forest. An interesting point which emerged during the present work was that on cold evenings when the catch of *A. africanus* was much diminished, that of *A. gambiae* was usually considerably increased, except in cases where wind (which puts an end to biting activity in all species) was the immediate cause of the fall in temperature. Towards the end of the series we came to recognise that on evenings when *A. gambiae* was at once taken in numbers, the catch of *A. africanus* would be correspondingly small.

The suddenness with which the main biting activity of *A. africanus* begins and ends can be explained only on the supposition that light intensity is the controlling factor. For light is the one factor that changes rapidly enough at sunset to explain the abrupt beginning and end of the biting-peak which corresponds to the period of deep twilight and often lasts for half an hour only. It must be recognised, however, that temperature may have a very important secondary influence. Low temperature may partly or even completely inhibit biting activity. Further, at dawn, when low light intensities again occur, no definite peak of activity is shown by this species in Bwamba. This is almost certainly on account of the low temperatures prevailing during this period.

The Biting-habits of *A. africanus* in Captivity.

In the hope of transmitting yellow fever to a susceptible animal by bite, the *A. africanus* taken in the field were given the opportunity of engorging on a non-immune rhesus monkey in the laboratory before being triturated for inoculation into another rhesus.

The monkey was attached by its collar and by bandages to a padded monkey-board. It is to be admitted that we have not as yet seen a board of ideal design. In most cases the monkey is able to make movements which disturb the feeding mosquitoes and a good deal depends on choosing a tranquil rhesus—preferably a female—for work of this type.

In considering the results given below, it is necessary to deduct from the total catch the small number of mosquitoes that died or were lost before they could be given an opportunity to bite, in other words, to consider only the mosquitoes available for the experiment.

During the first 2 weeks, small cages made of mosquito net were used to confine the mosquitoes during the time allowed for biting. Of 185 *A. africanus* available during this period, only 102 (55 per cent.) could be induced to bite, and only 60 (32 per cent.) actually engorged. As this result was considered unsatisfactory, the method was changed and during the following 9 weeks each mosquito was fed individually in a short wide tube. The great majority were fed on the monkey's nipples, a bare and vascular area very suitable for rapid work. Feeding was carried out inside a large " dining-net." As soon as one mosquito had begun to feed, the tube was removed and another was started on the opposite side of the monkey. When engorgement was complete, the mosquitoes flew to the sides of the net, where they were recaptured by an assistant.

A very noticeable feature was the remarkable speed with which *A. africanus* engorged. In most cases the proboscis was inserted almost at the moment of alighting, and feeding was very often completed within half a minute. It would have been impossible to feed so many mosquitoes individually in the time available had this not been the case. Mosquitoes which refused to bite were always given a second opportunity after about half an hour, but only a very small percentage of these utilised this further chance to feed. In general, if a mosquito did not bite at once, it did not bite at all.

This method proved to be highly satisfactory and, of 3,323 *A. africanus* available for biting during the 9 weeks of the experiments, 2,883 (87 per cent.) bit the monkey and 2,808 (85 per cent.) engorged fully. These are seen to be high figures, when we take into consideration the large size of the sample, and the facts that the mosquitoes were of all ages, that some had been damaged during capture or transit, and that some had already engorged (or partly engorged) on the catchers. The results were consistent from day to day. While it seems unnecessary to quote at length the daily figures for the 9 weeks, the weekly totals may be given.

Week			Mosquitoes available	Number biting		Number engorging	
3	...	...	...	113	87	(77%)	78	(69%)
4	...	...	...	104	89	(86%)	87	(84%)
5	...	...	...	226	191	(85%)	187	(83%)
6	...	...	...	280	223	(80%)	218	(78%)
7	...	...	...	224	199	(89%)	196	(88%)
8	...	...	...	539	464	(86%)	448	(83%)
9	...	...	...	756	656	(87%)	647	(86%)
10	...	...	...	535	493	(92%)	479	(90%)
11	...	...	...	546	481	(88%)	468	(86%)
Totals	...	...	3,323	2,883	(87%)	2,808	(85%)

During the course of the work we noted that there were always some engorged or partly engorged females in the catch. The catchers were therefore warned of the necessity of collecting the mosquitoes as soon as they alighted to bite because specimens already containing blood were unsuitable for feeding on a monkey next morning. Our attention was then drawn to the very silent and rapid approach of *A. africanus* to its host, the remarkably painless bite it inflicts and the speed with which it becomes engorged. It was thus confirmed that the habits observed in captivity are also characteristic of this mosquito in its natural habitat.

The Relationship between *A. africanus* and *A. luteocephalus*.

Aëdes (Stegomyia) africanus, Theo., and *A. (S.) luteocephalus*, Newst., are obviously closely allied and stand somewhat apart from the other African *Stegomyia* spp.

The larvae are differentiated from others of this subgenus by the fact that their combs are composed of spatulate spines with apical fringes. All other species have pointed spines, without apical fringes (Hopkins, 1936). If Hopkins is followed, the larvae of *A. africanus* are easily differentiated from those of *A. luteocephalus* by minor characters. Past work in Bwamba, however, has shown that an adult typical of *A. africanus* may emerge from a larva which had been assigned without hesitation to *A. luteocephalus*. It was this that first suggested that the differences between these mosquitoes might not be great enough to warrant full specific rank for *A. luteocephalus*.

Study of the present large sample tended to confirm this view. Typical adult females are easily distinguished, mainly by the large amount of gold in the cephalic and thoracic pattern of *A. luteocephalus*, and also by the fact that this mosquito has a banded abdomen, whereas *A. africanus* has not. Among the Mongiro and Mamirimiri females, however, we found a long series of intermediate forms, with the amount of gold on head and thorax and the length of the central thoracic line varying considerably. Further, we took a few specimens with approximately " *africanus* " head and " *luteocephalus* " thorax, and *vice versa*. As all the specimens lacked abdominal banding, all were assigned to *A. africanus*. It may be mentioned here that in subsequent work one of us (A. J. H.) has taken specimens of *A. africanus* in the Semliki Forest which did have abdominal banding, though otherwise typical, and that on 2 occasions typical *A. africanus* and typical *A. luteocephalus* have been taken biting in the same tree on the same night.

Some further points of resemblance between *A. africanus* and *A. luteocephalus* which may be mentioned are as follows :—Edwards (1941) describes the male terminalia of *A. luteocephalus* as " hardly differing from those of *africanus*." Both breed in tree-holes, both bite in trees and both (Kerr, 1933) have similar biting-cycles. Even the differences in adult pattern (apart from abdominal banding) are of degree rather than of kind.

The position is therefore that the essential diagnostic characters of these mosquitoes are based on minor larval differences which we have not found entirely reliable, and on adult pattern and coloration, characters always to be regarded with reserve where doubtful specific differences are concerned. More particularly in the case of such a variable subgenus as *Stegomyia* and all the 7 species with which we are familiar are exceedingly variable, we doubt whether the characters mentioned are adequate for full specific distinction, especially as intermediate forms have been found.

The main possibilities seem to be that *A. africanus* and *A. luteocephalus* represent the extreme forms of a very variable species, or (improbable) that they are 2 very closely allied species, hybridising where their distributions overlap or, as seems most likely, that the distinction should be relegated to the rank of a variety or at most a

subspecies. In this case *A. luteocephalus* (first described in 1907) would rank as a variety or subspecies of *A. africanus* (first described in 1901).

The point is not an academic one, as both are capable of transmitting yellow fever under laboratory conditions (Bauer, 1928 ; Philip, 1929) and furthermore, though overlap occurs, *A. luteocephalus* is found in large tracts of fairly dry and open country where the essentially sylvan *A. africanus* is not known to be present.

Animal Inoculations.

At first mosquitoes were sent alive to Entebbe for inoculation at the Yellow Fever Research Institute, but it was soon found preferable to carry out the entire work in Bwamba.

Four groups of mosquitoes, *Aëdes africanus*, " other *Aëdes* ", *Taeniorhynchus* spp. and *Aëdes aegypti*, were kept for inoculation. The remaining genera were discarded, as space for housing rhesus monkeys in mosquito proof quarters was limited. *A. africanus* were inoculated daily, the other groups twice weekly. Mosquitoes which died before the day for inoculation were kept in a refrigerator and subsequently inoculated along with the others, which were maintained in the meantime in small Barraud cages. *A. aegypti* were inoculated into mice only.

Immediately after being killed with chloroform, the mosquitoes were triturated in small sterile mortars, without the use of abrasive, and the suspensions were made in 10 per cent. non-immune human serum in physiological saline. The quantity of serum-saline varied from one lot to another, but was kept as small as possible. In general 1·0 to 1·5 ml. was used, and only occasionally was 2·0 to 2·5 ml. necessary. The suspensions were spun for 5 minutes in a hand centrifuge and the supernatant fluid was then withdrawn in a syringe and, without filtration, injected subcutaneously into a rhesus monkey, or intracerebrally into mice.

One rhesus monkey was set aside for inoculations of *Taeniorhynchus* spp. This monkey received 865 mosquitoes in 19 lots. Another rhesus received 578 " other *Aëdes* " in 19 lots. Six monkeys were used (in daily rotation) for *A. africanus*, to minimise the dangers attendant on rapidly repeated inoculations of unfiltered material. In all, 63 lots, totalling 3,110 *A. africanus*, were inoculated in Bwamba. During the course of the work, which comprised the inoculation of 4,553 mosquitoes in 101 lots, 2 of the monkeys developed non-fatal fevers of unknown origin. In neither case, however, could the fever be attributed directly to the effects of inoculation. The *Taeniorhynchus* monkey at one stage developed an abscess directly attributable to inoculation, but this healed rapidly after aspiration. It is to be noted that mosquitoes belonging to this genus seem to be particularly prone to cause abscess-formation when inoculated into experimental animals. These results show that centrifugation without filtration is a method which can be used in the field with a reasonable degree of safety, even where repeated inoculations are to be made.

Summary.

In an attempt to isolate yellow fever virus from *Aëdes* (*Stegomyia*) *africanus*, Theo., an intensive series of catches was carried out during 11 weeks of 1945 in 2 forest areas in Bwamba County, Uganda. Most of the work was carried out on platforms in trees during the sunset period, since the main biting activity of *A. africanus* occurs in the forest canopy just after sunset. Virus was not obtained during the course of this work.

The results confirmed that *A. africanus* is the dominant arboreal Culicine of the sunset period. In catches carried out actually in the canopy it formed 69 per cent. of the total Culicines taken.

Other species particularly common in the canopy in the sunset period were *Taeniorhynchus* (*Mansonioides*) *africanus*, Theo., *Anopheles* (*Myzomyia*) *gambiae*, Giles, and the Tabanid, *Chrysops centurionis*, Aust.

A result of particular interest was the capture of 2 *Aëdes* (*Stegomyia*) *aegypti*, L., in the trees of the one study area, and of 13 actually in the forest canopy of the other. Both areas are primary rain-forest, and native huts are very scarce in the vicinity.

It is pointed out that *A. africanus* is peculiarly susceptible to the influence of weather conditions, and that low temperatures may partly or even completely inhibit its biting activity in the sunset period.

The methods of feeding *A. africanus* on rhesus monkeys in captivity are described. The remarkable speed with which this species engorges is discussed.

The close relationship between *A. africanus* and *A.* (*S.*) *luteocephalus* is discussed. The writers believe that the differences between these mosquitoes do not warrant full specific rank for *A. luteocephalus*, more particularly as intermediate forms have been found.

The methods of inoculating triturated mosquitoes into laboratory animals in the field are described.

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THE CLIMATOLOGY OF BLOWFLY MYIASIS.
II.—OVIPOSITION AND DAILY WEATHER INDICES.

By John MacLeod.*

Ministry of Agriculture Veterinary Laboratories, Weybridge.

Contents.

Introduction.

In the first paper of this series (MacLeod, 1947), observations on the degree of response of blowflies (*Lucilia sericata*, Mg.), to a standard attractant under different conditions of temperature, sunshine, humidity and evaporation were described. The observations were critical, in the sense that exposures were for one hour only, so that the meteorological conditions were fairly constant throughout the test. Temperature appeared to be the primary influencing element, the correlations obtained between oviposition and other elements being in all cases spurious. There was some evidence, however, of a true, and negative, association with insolation, and a suggestion of a similar association with humidity. The significance of air-movement was not examined, and the greater part of the total variability was found to be due to sources other than the three tested elements, temperature, insolation and vapour tension.

The extent to which daily records—the total oviposition over 24 hours, and the daily weather as described by the usual meteorological indices—can be used as a measure of the fundamental association of temperature and oviposition has now been examined. The examination is based on tests carried out over a period of five years.

It is unlikely that an exact relation could be discovered with any of the indices. They are extreme values, or they average or sum the conditions for the day. They may have little relation to the conditions obtaining at the time of oviposition, and thus there will be an inevitable blurring of the picture. Further, observed associations cannot be claimed as cause and effect, and the analyst of such data must discipline himself to the outlook that the apparent causal relation he is studying is only a more or less imperfect reflection of a relation between an unknown causal complex and oviposition; the degree of correspondence between variations of the unknown and of the index studied cannot be measured.

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*Research Fellow of the Animal Health Trust.

Nevertheless, such indices are most commonly available and oviposition records will usually be for day-units. Associations between them must therefore be examined as possible measures for the field ecologist's use. For the immediate purpose of this study, their isolation is a necessary preliminary to their comparison with those for naturally occurring oviposition, as indicated by strike records ; differences obtained should be an indication of conditions critical for development of attraction and for viability of deposited eggs.

The experimental data. The meteorological station, situated on the experimental farm, consisted of a Stevenson screen housing maximum, minimum and wet-and-dry-bulb thermometers, a black bulb *in vacuo* sun thermometer, a 5-inch standard rain gauge and a standardised Livingston white atmometer sphere with rain valve attachment. Readings were made at 9 h. official time, because of administrative difficulties in using G.M.T.

Temperature readings were in degrees F., and in the previous paper this scale was used. Whilst the undesirability of having different scales in successive papers is admitted, the centigrade scale has been used here so as to conform with later work.

The oviposition results have been compiled from the controls for experiments on protection against blowfly, carried out at the Cooper Field Research Station, West Herts., between 1936 and 1940. The attractant used was in all cases 4 per cent. ammonium carbonate. The sheep were attractivated in the forenoon, and the results read in the following forenoon. Results were entered as positive or negative, no account being taken of the number of egg clusters per sheep. Culture tests were not made, but there is reason to believe (MacLeod, 1943, 1947) that the flies concerned were in the great majority of cases *L. sericata.*

Since the meteorological readings refer to the period 9 h.–9 h., there is a possible source of error in that oviposition occurring between 9 a.m. and examination of the sheep one to three hours later would be ascribed to the preceding day. Since oviposition occurs mostly in the afternoon (MacLeod, 1947) and since the attractant must be weakening after a day's exposure, it is hoped that this source of error is not serious.

The distribution of test days is shown in Table I. There was some degree of selection of actual days, in that, during an experiment, testing might be omitted on a morning of heavy rain which gave no promise of lifting, but otherwise the tests were randomly distributed in that they were determined by the work programme and the availability of experimental materials.

TABLE I.

Distribution of test days.

Year	June	July	Aug.	Sept.	Oct.	Total test days	Positive days
1936	5	7	1	—		13	12
1937	—	1	16	14	21	52	32
1938	—	—	3	13	19	35	21
1939	1	10	1	11	3	26	25
1940	4	—	4	5	—	13	13

Meteorological Indices.

Five indices of daily temperature conditions have been used.

DAILY MAXIMUM TEMPERATURE (T_M), the maximum screen temperature reached in the period 9 h. to 9 h. This is usually registered in the afternoon, about 15 or 16 hours B.S.T. (Bilham, 1938).

Daily Minimum Temperature (T_m), the corresponding lowest temperature recorded—usually reached in the early morning, before or about dawn.

Maximum Temperature in the Sun (T_S), which may be either in the forenoon or afternoon.

Average Air Temperature (T_A). The mean of T_M and T_m has been taken as a rough estimate. Its relation to the mean of hourly temperatures is not known, and little use has been made of the index in this paper.

Solar Radiation (S.R.) obtained from the daily differences of T_S and T_M. The difference does not necessarily represent the daily maximum value of the S.R. factor, or insolation, discussed in the earlier paper, since the T_S and T_M may not have been coincident. The index will, however, never exceed and will sometimes be an underestimate of the maximum difference between temperature in the sun and the air-temperature at that moment.

There were no sun temperature records for 1936. Occasionally, breakdowns or other causes resulted in the sporadic omission of individual records of all indices ; this accounts for the apparent discrepancies in totals of test days.

Daily moisture records were also in five indices. All relate to the period 9 h. to 9 h.

Relative Humidity (R.H.), a single daily reading taken at 9 h. official time. This is a reasonably reliable representation of the daily mean R.H. during B.S.T. months, but tends to be high, the diurnal curve reaching the mean level about 9 h. G.M.T. The following are the figures for Kew (M.O. 421, 1938)—

	July	August	September	October
8 h. G.M.T.	76·1	80·7	85·0	89·4
9 h. G.M.T.	70·9	74·8	79·8	86·1
10 h. G.M.T.	67·3	70·3	74·7	82·6
Day Mean	73·0	75·8	79·2	84·6

These values are, however, averages for the month, and during these months the daily fluctuation is at its maximum. A single daily R.H. reading, as an estimate of that particular day's R.H., is, therefore, liable to be frequently and grossly misleading.

Precipitation (P.), the total rainfall, in mms. The P. distribution is extremely skewed, with a very high zero frequency. The mean of the observed records is 1·5 with variance about 16. Log values (of N+1) are used, to foreshorten the distribution.

Evaporation (E.), the total evaporation in ccs. from a Livingston white atmometer sphere. Because of the danger of night frosts, which crack the atmometers, readings were usually discontinued before the cessation of tests, so there is probably a slight temperature bias in the E. population sample, as compared with the other samples.

From these three indices two others were calculated, saturation deficit and the P/E Ratio.

Saturation Deficit (S.D.). An estimate of the daily mean S.D. was calculated from the 9 h. R.H. value and the T_A ($\frac{1}{2}\overline{T_M + T_m}$). The daily mean S.D. during July (Kew) coincides with the hourly S.D. usually between 8 h. and 10 h. G.M.T. It has been shown by Prescott (1931) for South Australia that the S.D. calculated thus gives a good approximation for the mean monthly S.D. The applicability of this formula has not so far as I am aware been worked out for this country. The estimate of daily S.D. used here must, therefore, be recognised to be very provisional, particularly for application to individual daily values.

THE P/E RATIO (P/E).   As will be shown later, no single moisture index is of much value in relating oviposition to the daily weather picture.   The moisture factor in surface ecoclimates, in so far as it affects such almost subaerial forms as the blowflies, is more likely to be some complex resultant of available moisture and air-drying-power, i.e. of P, run-off, and E.   Various attempts have been made to assess the " efficiency " of rainfall by integrating in some way the precipitation and rate of water-loss.   The rainfall-minus-evaporation, used for monthly totals, was tentatively applied by Livingston (1913) to the delimitation of plant climatic areas in the United States.   For the ecologist, however, present-day atmometer values cannot yet be translated into absolute terms of water loss, although recent work by Livingston (1946) with his atmometer is promising.

The de Martonne formula, $P/T+10$ (Andrews & Maze, 1933) adjusts to some extent the monthly rainfall values in respect of the resulting availability of soil moisture.   An improvement of this, for aerial conditions, in that it uses the drying power of the air, is the Meyer ratio, P S.D. (Prescott, 1931).   Transeau (cited by Livingston, 1913) using Piche evaporimeter records, applied the ratio P/E to a study of the forest zones of Eastern America.   Davidson (1935) showed that the ratio P/E for monthly values gave similar results to the Meyer ratio, in delimiting bioclimatic zones for plant growth in Australia.

Though the ratio of monthly precipitation to drying power of the air is probably a characteristic of a locality, the variations from the mean within a month can usefully be applied to a study of the variability of the weather.   For such day-to-day records the components of the air-drying-power may vary widely in relative importance, and so the more general measure, E, is preferable to the S.D.   For calculating daily P/E values, two adjustments were found necessary, so as to obtain finite ratios, viz., zero rainfall has been taken as one-tenth the minimum recordable amount, i.e., 0·01 mm., and minimum daily evaporation as 1 cc.   If a probable maximum E value of 100 be assumed, there is thus a minimum P/E value for any day of 10^{-4}.   The calculated values were, therefore, multiplied by 10^4, to give workable figures.   The log values, grouped in classes of 0·250 (0·251–0·500, 0·501–0·750 ... 5·251–5·500) have the following skewed but not excessively abnormal distribution :—

1, 22, 16, 10, 2, 4, 8, 5, 3, 5, 6, 2, 4, 4, 4, 3, 2, 6, 1, 1, 1.

Some Analytical Considerations.

The experiments which yielded the present data were not designed for this study ; the data are, therefore, unbalanced and in many respects inadequate.   Possibly the principal objection from the analyst's point of view is the variable numbers in the test groups—the original control groups.   The number varied considerably, usually in proportion to the number of experimental animals ; although three-quarters of the tests had group sizes between 4 and 10, the extremes ranged from 1 to 30.   Were the numbers of gravid female flies low, an important source of variation in the results would thus be introduced.   Table II gives the distribution of group sizes, and for each

TABLE II.

Departure from the mean (41) with different sizes of test groups.

Group size	1,	2,	3,	4,	5,	6,	7,	8,	9,	10,	12,	13,	14,	16,	18,	20,	25,	26,	30
Frequency	1,	2,	3,	5,	31,	9,	4,	12,	18,	25,	1,	1,	1,	2,	17,	2,	2,	2,	1
Average per cent. positive	95,	50,	33,	30,	27,	60,	70,	56,	43,	34,	85,	0,	25,	30,	43,	70,	75,	50,	85

Smoothed results :

| Positive anomaly ... | 18, | | 11, 21, | 7, | 3, 13, | | 7, 22, 24, 29 |
| Negative ... ... | 3, 11, | 2, | | 1, 4, 23, | 8 |

the average of the per cent. positives (which are recorded to the nearest 10 per cent.). The mean per cent. positive of the 139 tests is 41·33.   The curve of average positives

per group size was smoothed in unweighted running means of three, and the departure of each smoothed value from the grand mean—its anomaly—is entered in the table. The absence of a downward trend with increasing group size suggests that little if any of the dispersion of results need be attributed to insufficient available flies for the larger group sizes.

The scatter of group size has, however, another effect, which is important. If the oviposition rate per test is not weighted for the size of the group, then equal value is being given to results from tests of widely different levels of accuracy. If the rate per test is attributed to each sheep in the group, then the results are not independent observations.

Analysis is further complicated by the lack of uniformity in the scatter of the tests, in relation to weather and time factors. As a consequence, observed differences in year to year or seasonal comparisons, or apparent intereffects of simultaneously tested meteorological variables, cannot be satisfactorily assessed by the ordinary statistical methods. For the greater part, therefore, the analyses are perforce confined to inspection of results tabulated for two or three criteria.

Effect of year and season. It was felt desirable, before relating the variability of the oviposition rates to weather, to examine whether it might have arisen from other than meteorological causes, namely, the annual and seasonal sources of variation inherent in the data. The analysis was made in triplicate, using for the meteorological factor the T_M, T_S and P/E indices respectively. The analysis with the T_M data is discussed in detail, in relation to certain statistical problems involved. For the reader who may wish to skip this rather technical statistical discussion, the ecological conclusions may be summarised thus :—

There is no evidence that season, *i.e.*, summer as against autumn, affected the relation between temperature, or P/E, and oviposition.

There were differences in the level of oviposition, in different years, in response to given values of T_M and of P/E. The irregularities between years for response to T_S were not significantly different from those to be expected from chance variations.

There was no proof from the analysis that the response to any of the three meteorological factors differed in type, as distinct from degree, in different years. The 1937 response to T_M, however, was suggestively different from that for other years.

Discussion of the meaning of these year differences in level, and possibly in quality, of oviposition response to individual factors is postponed to the final section, where they are shown to fit in with a suggested hypothesis of weather effect.

Analysis of year and season effects. For the T_M analysis, the results, expressed as percentages, were grouped in 5 categories of temperature, and divided into summer (June to August) and autumn (September/October) categories for each year. Table III summarises the data ; the mean percent. oviposition in each subclass, weighted according to the numbers of sheep used, and the number of sheep, are given. The figures in brackets are the unweighted mean and the number of experiments (see footnote, p. 186).

The table suggests the following conclusions : (1) There is, in different years, a wide variation in the mean incidence for a given temperature category. (2) This variation is not due to a seasonal effect at given temperatures, since, for all years, the grand means for summer and autumn show no consistent difference. For the five temperature categories the means are :—

Summer ...	—	53	68	60	51
Autumn ...	19	56	55	67	68

An attempt was made to estimate the year and season effects and to examine for interaction between temperature, year and season. The term interaction

TABLE III.

Oviposition mean percentages, for year, season and maximum temperature.

		−16°	17–18°	19–20°	21–22°	23°+
1936	S	—	52 33 (47 3)	60 20 (59 3)	56 16 (60 2)	79 28 (78 2)
	A	—	—	—	—	—
1937	S	—	26 31 (22 2)	48 33 (42 3)	12 8 (12 2)	31 87 (31 9)
	A	14 191 (14 21)	59 53 (54 7)	47 17 (46 2)	59 22 (58 3)	50 14 (50 2)
1938	S	—	83 12 (83 1)	90 30 (90 1)	—	75 8 (75 1)
	A	25 126 (24 25)	73 26 (73 5)	80 5 (80 1)	—	60 5 (60 1)
1939	S	—	68 40 (68 4)	76 37 (77 4)	100 17 (100 2)	79 28 (84 2)
	A	21 126 (21 7)	54 90 (54 5)	—	72 18 (72 1)	94 18 (94 1)
1940	S	—	53 61 (56 4)	58 12 (58 2)	53 32 (46 2)	—
	A	—	25 16 (25 1)	—	100 3 (discarded)	65 57 (57 3)

S=summer,   A=autumn.

First figure is mean of percentages ; second the number of sheep.

Figures in brackets are the unweighted mean and number of experiments.

describes intereffects where the changes produced are in kind of response. Those intereffects discussed in Part I and later in this paper, where the differences are merely in degree of response, correspond to a " main effect " of statistical two-way analysis.

The method used —a multiple classification analysis of variance (Snedecor 1946, p. 304 *et seq.*)—is designed to isolate the single factor and interaction components of the variance, but, as shown below, it introduced a number of difficulties, so that only presence or absence of main and interaction effects was determined.   Thus :—

(*a*) Because of the irregular nature of the data, there were missing subsquares in each of the three two-criteria tables constructed.   The temperature-year table has three missing squares.   Mean values for these were filled in from the formula for a missing plot in a randomised block layout, using the iterative technique described by Snedecor (p. 269).   Since the preliminary inspection of Table III suggested that the omitted factor, season, did not in itself affect the results, these interpolations were considered legitimate.

In both the year × season and temperature × season classifications, the omitted factor is important.   Each has one missing subsquare.   The " interaction sum of squares", from which the presence or absence of interaction is determined, is obtained directly without the need for estimating this missing value.   In the former case, however, the interaction mean square turns out to be greater than sampling expectation, and so the missing value is required before the main effects can be calculated ; filling in the missing square would, however, involve the assumption that the distribution of oviposition results is independent of temperature !

(*b*) There is a marked disproportion of subclass numbers. Special weighting methods for such " non-orthogonal " data have been evolved for two-criteria tables ; for a three-criteria analysis of this type, however, they must be applied to each two-way table separately, ignoring in each case the third factor. This may lead to startling discrepancies.

(*c*) The results are ratios, and involve low figures. Strictly, a " normalizing " transformation should have been used, since in their binomial form the data will not have completely independent mean squares for treatment and error variance, thus lessening the validity of the test for association. It was felt, however, that such transformation, with these essentially imprecise data, would have been a tedious supererogation.

The results of the analysis are given in Table IV. They confirm the conclusions from inspection—that the year effect is significant but not the season effect. There is no proof of interaction of year and temperature. That is, although the year of test affected the intensity of the mean oviposition response to each temperature category, the quality of the response did not vary significantly between years. There is, however, suggestive evidence of interaction, in that the 1937 curve of temperature response differs from the others ; the suggestion is developed later that there is in fact a true effect, though it is obscured in this analysis by the high variance of the data.

TABLE IV.

Main and interaction effects of temperature, year and season.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square
Total	1453052	137	
	Temp. × season		
Subclasses	494202	8	
Error	958850	129	7433
Temperature	352207	4	88052
(Corrected for interaction)			
Season (corrected)		1	6797
Interaction	15204	3	5068
	Year × season		
Subclasses	376030	8	
Error	1077022	129	8349
Interaction	105669	3	35223
	Temp. × year		
(Treatment effects calculated approximately from unweighted means)			
Temperature	8699	4	
Year	3094	4	774
Interaction	1393	13	107
Error (Mean square for individuals ÷ harmonic mean of sheep numbers)			190

When the total variance is divided between temperature and season, the inter-action component is again negligible, indicating that neither in itself nor as a vehicle for expression of the year effect did season affect the response to temperature. The year × season interaction is statistically significant, but the spurious origin of this

interaction is obvious from Table III, the omitted variable, the temperature of the test days, having a differential distribution between seasons.*

TABLE V.

Test for interaction between year and sun temperature.

Year	−40°		41–45°		46–50°		51–55°		56–60°	
1937	14·1	127	25·5	110	55·9	61	33·6	92	38	66
1938	1·9	53	33·3	45	62·4	27	78·4	79	75	8
1939	19·5	36	20·3	54	44·1	102	69	145	100	37
1940	10*		29	14	25	16	48·7	39	54·1	112

First figure is mean per cent. ; second is number of sheep.   * interpolated.

Total S.S.	12975	Df 18	
Temps.	8720	4	
Years	1244	3	Mean Square 415
Interaction	3011	11	274
Error (11104/36·14)			307

The data for temperature in the sun were also examined (Table V). There are irregularities in the level of oviposition rate, but these do not seem to be due to a definite intereffect of year on sun-temperature. The response to temperature, throughout its range, however, is apparently of a different pattern in 1937 from that for other years. The significance of these irregularities, *i.e.* the main and interaction effects of year on temperature, were examined from the mean oviposition rates,

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*Mr. D. J. Finney, to whom the above analysis was submitted, made the following comments :

   " On looking at your table [Table of basic data for T_M, year and season] once more, however, I consider that the experiment rather than the animal should be regarded as the unit.   Rough tests show that variation between means for different experiments classified in the same year, season, and temperature, is greater than would arise from a binomial distribution of affected animals.   Therefore, your weighting with ' f ' may be unsatisfactory, and I prefer to take the means from the 138 experiments as the basic data.   Of these, I propose to discard four based on 3 or less animals each, as these must be very unreliable, and then to assume that the remainder are all of equal accuracy."

The subclass means thus obtained are included in Table III, in brackets.

With regard to the actual analysis, he considers that, because of the danger of misleading results when ignoring an important factor, the above method is unsatisfactory for use with disproportionate frequencies, and a full analysis for the three factors simultaneously would be required. There is apparently no published account of such an analysis.   In this case it is clear from inspection that there is no consistent difference between summer and autumn, and so, ignoring season, Finney has applied the method of fitting constants to examine more exactly the year and temperature effects and intereffects.   He obtained no evidence of interaction, and states :

   " Thus temperature effects may be regarded as consistent from year to year, except of course for an overall shift in level of incidence from year to year.   The mean for all the data is 41·8 per cent. and separate means for the different temperature ranges, after the elimination of year differences, are :—

−16°	17–18°	19–20°	21–22°	23°+
17·9%	55·0%	61·6%	59·3%	56·6%

There is some suggestion of a rise to max. incidence at about 20°, but in fact only the large difference between the lowest range and the others can be regarded as established ; from 17° upwards the differences are too small to be estimated satisfactorily without far more extensive data.

   Possibly it is of interest also to record the means for the five years, freed from complications due to temperature :

1936	1937	1938	1939	1940
42·7%	30·2%	51·0%	54·0%	36·6% "

using the sheep-weighted data but without weighting for disproportion of subsquare frequencies. The error mean square for individual results was reduced to the means level, as before, by dividing by the harmonic mean of the sheep frequencies.

The analysis suggests that the interaction component of the variance would almost certainly be found to be no greater than sampling expectation. It also reveals no significant discrepancy between the degree of response to sun temperature in different years.

Table VI gives the means of the weighted oviposition rates in each subclass for year and four intervals of the P/E ratio ; each subclass is subdivided for summer and autumn. There is again no consistent seasonal difference in the oviposition rate. The annual results show main effects for both year and P/E. The year-on-P/E inter-effect, *i.e.* the effect of year on response of oviposition to P/E, is fairly consistent for all P/E categories ; similarly, the ranking of P/E categories is fairly consistent for different years. If these two impressions be accepted, it follows that there is no interaction between the two factors.

TABLE VI.

Oviposition in relation to P/E, year and season.

Log (P/E × 104)		to ·75		·76–1·50		1·51–3·0		3·04 +	
Year	Season								
1936	S	73	11	70	10	63	43	55	33
	A	—		—		—		—	
1937	S	36	67	38	16	41	53	0	13
	A	31	32	51	73	45	31	40	10
	Total	33	99	50	89	43	84	17	23
1938	S	90	30	83	12	—		75	8
	A	—		46	37	41	46	28	65
	Total	90	30	55	49	41	46	33	73
1939	S	—		95	18	53	36	—	
	A	85	39	—		89	55	43	28
	Total	85	39	95	18	75	91	43	28
1940	S	—		97	28	40	32	25	16
	A	87	15	58	52	33	6	35	32
	Total	87	15	71	80	39	38	32	48

The first figure is the mean per cent. oviposition ; the second is the number of sheep.

Methods of analysis of meteorological effects. Were the tests proportionately distributed over the range of each factor, one could apply the above method to examine the intereffects of meteorological conditions. When meteorological data are used, however, there is a further source of disproportion, additional to the irregularity of distribution already encountered. Many of the meteorological factors are themselves correlated. If a pair so related are being examined, their simultaneous related variations tend to make the observations lie on a diagonal ellipse across the two-way table, few or none occurring in the remaining two corners. This increase in disproportion of frequencies and in number of missing subsquares precludes use of the multiple classification analysis of variance.

The method of partial regressions used in the first part of this series serves to isolate the fundamental relationships of each individual factor, under the hypothetical conditions of absence of variation by the other factors considered. The present study

is more concerned with checking the reliability of observed relations of individual factors, and of observed intereffects. Significant associations can be used, say for comparing oviposition rates obtained under different treatments at different times ; their value for this is not necessarily lessened if they happen to be essentially spurious in origin. Intereffects between correlated factors, such as the partial correlation of each, are similarly of practical interest only when they occur within the natural limits of relative variation.

The requirements of the analysis can be met, to some extent, by determining the total associations of individual weather indices, and separately examining the nature of observed intereffects. Total associations can be tested for significance by analysis of variance. By an extension of the analysis the character of the association can be tested as to whether it is a straight line regression slope or otherwise. If the regression is linear, the degree of association can be expressed by a correlation coefficient. With non-linear association, the correlation coefficient will undervalue the degree of correlation ; a significant value in such case will at least indicate a lower limit to the degree of association.

It is to be understood that with records of this character the inference even from linear correlations is limited ; a correlation implies merely that, associated with the occurrence of the tested index—say, a given max. sun temperature—there could be expected *during the course of that day* a fixed value of some factor, or of a complex of which the T_s may not necessarily even have been a part. Variations in this unknown variable and in oviposition tended to keep in step, with a degree or precision only the lower limit of which is indicated by the coefficient r for T_s and oviposition.

For examining intereffects, inspection of tables of two or three indices will reveal whether oviposition is affected by one factor under constant conditions of the other. Also, by examining the different combinations of pairs of indices of weather, it is

TABLE VII.

Effect of related variation of two environmental factors.

	True Associations			Observed Associations		
	AB	YA	YB	Total YA	Total YB	Interaction
1	+	lin.	0	True association not affected.	Positive, lin., spurious.	
2	—	,,	,,	True association not affected.	Negative, lin., spurious.	
3	+*	lin.	+lin.	lin., b and r increased.	lin., b and r increased.	
4			—lin.	lin., b and r reduced.	lin., b and r reduced.	
5	—*	lin.	+lin.	As for row 4		
6			—lin.	As for row 3		
7			0	True association not affected.	Non-lin., spurious.	B on A, spurious.
8			+lin.	Curve partially straightened.	Curve departs from linearity.	B on A, spurious.
9	+ non-lin.		—lin.	As for row 8		
10†			non-lin.	Curve altered.	Curve altered.	B on A & A on B. Both spurious.
11	— non-lin.			As for AB+.		

Lin. = linear. b and r = coefficients of regression and correlation.
    * With non-linear correlation of A and B, the observed total associations YA and YB are non-linear, reflecting the AB association curve.
    † If the true regression curves are of similar character, the observed regression curve is an exaggeration ; if they are complimentary it is a compromise.

usually possible to determine whether observed associations with single indices are genuine or a spurious effect of related variation of the indices themselves. The interpretation of two-way tables where such related variation occurs will be discussed in detail in a paper now in the press ; Table VII, abbreviated from this discussion, summarises some relevant effects of such correlation on observed single linear and non-linear associations.

Oviposition and Temperature Indices.

For the tests of total association, correlation tables were set up. The oviposition results were expressed as percentages, and each was weighted by the number of sheep in the test group. This " fy " value was entered in the correlation table, but the unweighted frequency of experiments was used for the degrees of freedom for analysis of the variance, and for calculating the standard error of correlation coefficients. The oviposition ordinate was kept in units of 10 per cent. Table VIII summarises the analysis of variance of results for the different classifications. It has already been shown, as incidental to the study of the influence of year and season, that the T_M and T_s indices are associated with oviposition. They are included in this analysis as a check on the effect of reducing the error variance through the coarse grouping of the oviposition percentages.

TABLE VIII.

Analysis of variance with single indices.

Index	Tests	Treatment		Error		P. for no association	Devs. from Regression		P. for Linearity
		Mean Sq.	Df.	Mean Sq.	Df.		Mean Sq.	Df.	
T_M	139	328	18	66·9	120	$<1\%$	161	17	$<1\%$
T_m	123	127	17	87·8	105	$>5\%$			
T_s	125	657	7	73·9	117	$<1\%$	53	6	$>5\%$
S.R.	125	168·8	30	87	94	$<1\%$	53·5	29	$>5\%$

The differences in the estimates of variance represented by the mean squares for error are probably due largely to the fact that they are based on different samples, through irregular omission of individual index records. Their limited size suggests a close relation between the indices ; otherwise the estimates of error would have been disturbed by the inclusion of differential omitted effects.

The results indicate a significant association of oviposition with three of the indices. The T_m effect fails to reach significant level, and thus the T_M index is probably a better measure of the unknown variable controlling oviposition than the T_A index.

Regression on Air-Temperature. The temperature (actual), and oviposition relationship, in the range 14° to 22°C., is exponential (see MacLeod, 1947), but is not so with daily values of T. The character of the relationship, with this type of data, would not be expected to be clearcut, but it is interesting that in each year except 1937 the regression curve appears to become horizontal at 18° to 20°C. This may be due to distortion by a correlated factor which has itself a non-linear relation with oviposition (Table VII, row 8) ; alternatively, it might be interpreted as indicating

that at air temperature maxima below this the daily maximum follows sufficiently closely the variations of the unknown complex controlling degree of oviposition per day as to reflect to some extent the regression of oviposition on this complex ; at higher maxima the relationship becomes obscured or lost.   Fig. 1 shows the smoothed regressions (running means of three) on T_M for all results less 1937, and for the 1937 results.   The T_A curve also shows a division into two phases.   (Compare also the total results when freed of year effects, as given by Finney.)   As a rough measure of the significance of the difference in regression shape for 1937, the correlations for separate years were calculated and compared for homogeneity.   It is to be noted that because of the non-linear association, the coefficients have no meaning other than as mathematical symbols by which homogeneity is tested ; they do not measure the degree of association.   The two independent air temperature indices T_M and T_m showed a marked discrepancy for 1937, thus :—

	All years	1937	1938	1939
T_M	·477 (139)	·354 (52)	·643 (35)	·755 (26)
T_m	·164 (123)	—·289 (35)	·342 (35)	·457 (26)

The figure in brackets is the number of pairs of observations.   The three annual estimates for each index were tested for likelihood that their differences were merely sampling fluctuations.   The r values were transformed to Z^1 values and weighted for observations ; the χ^2 test of homogeneity of these gave probabilities for T_M and T_m of less than 1 in 20 and less than 1 in 100 respectively.   The 1937 temperature association would thus appear to be definitely different from that for other years. As will be shown later, the effect of the P/E factor on the temperature relationship affords a possible explanation of the apparent optimum of 18–22° for 1937.
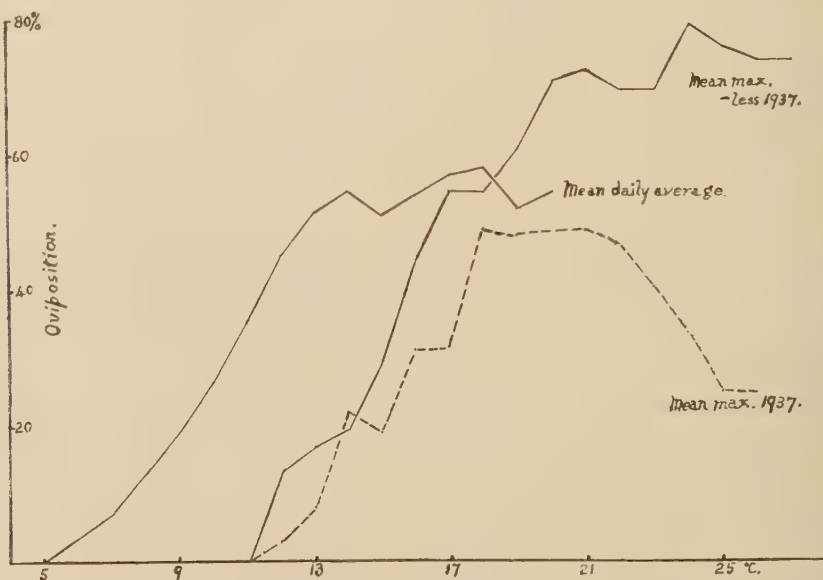
Fig. 1.—Regression of percent. oviposition on temperature showing the change of curvature at 20° (T_M), or 13°(T_A), and the abnormal regression curve for 1937, with a lower flexion point and lower optimum temperature.

Lower critical limit of temperature. In the work already reported (MacLeod, 1947), the temperature threshold for oviposition was taken as 14°C. ; oviposition at lower temperatures was, however, recorded. From the present survey of field tests it appears that the incidence at temperatures below 14° is higher than the 2 per cent. assumed ; on days when the maximum temperature reached only 12°C. and 13°C., 3 of 36 and 23 of 132 exposed sheep respectively were blown.

These results are interesting in view of the finding by Nicholson (1934) that flight activity of *L. sericata* is of a very low order at temperatures of 15°C. or below, though general activity, *e.g.* crawling or running, can occur at lower temperatures. Mellanby (1939) also gave 14°C. as the temperature at which flight became possible for this species. With both workers, insolation was excluded by the test conditions, and it is possible that despite the contrary findings, this factor may be responsible for depressing the threshold of air temperature under natural conditions (MacLeod, 1947).

Maximum and minimum temperature intereffects. The maximum and minimum temperatures, though fairly closely correlated, are not exactly so, and the effect of different immediately preceding minima on the oviposition for a given maximum can be examined from the dispersion. 1937, a " poor " year, and 1939, a " good " year for oviposition, were thus examined, and neither showed any effect of low night temperature on activity, or any influence of T_m on the lower critical limit of daily T_M.

The oviposition × insolation association. The S.R. index has a positive, and linear, association with oviposition. The degree of correlation is quite high. $r = \cdot 52$ for 125 pairs of observations. The T_s correlation is even closer ($r = \cdot 57$). The actual association (total) of SR has been shown to be negative (MacLeod, 1947). In the present observations, of course, the SR and oviposition coordinates were not necessarily synchronous in occurrence, but the complete reversal is interesting. The straight line character of the regression on daily max. insolation is unexpected, since the partial association with actual insolation, for fixed temperature, is apparently negligible (pt. I), and the regression curve of daily T_M is non-linear, and roughly unimodal (*cf.* Table VII, row 7). Admittedly the correlation of S.R. and T_M appears to be non-linear (see Table II), there being a correlation trend to about 18–19°C., but apparently no increase of S.R. for subsequent increase of T_M. A spurious observed association with S.R., due to related T_M variations, would still, however, have the general character of the observed regression on T_M. This is non-linear (fig. 1). The observed association of the S.R. index would thus appear to be met only by the conditions of row 10 of Table VII, *i.e.* that there be a true and non-linear association with S.R. In passing, it may be remarked that the observed B regression of row 10 is apparently linear, for a linear AB association, if the true B association is a hollow regression curve, and the A association a similar but reversed, *i.e.* modal, curve.

Temperature × insolation intereffects. In the two-way table of percentage oviposition for S.R. and T_M, (Table IX), the consistently negative results extending down and to the left of the table have been omitted to save space, and so the correlation of S.R. and T_M is not clearly seen in the table. The scatter of values is such that the character of the partial regressions cannot be satisfactorily determined, but the table serves to demonstrate that the partial association with T_M, *i.e.* for fixed S.R., is greater than that for S.R. Thus, of the 14 rows from which a trend could be detected, if present, 9 show a positive correlation with T_M. Of the 15 similar columns, only 6 show positive correlation with S.R. ; in the remainder the trend is obscured by the irregularity of the results.

It is clear, however, that, as an overall effect, oviposition rate tends to be higher for given temperature ranges, with higher S.R. maxima. This is shown again in

TABLE IX.

Insolation × temperature intereffects.

S.R.	13	14	15	16	17	18	19	20	21	22	23	24	25	26
38					83	65			100					
36			80		50	89	75		100	100				
34	33		17	83	80, 70	44, 100	0, 53	83, 33, 66	90	58, 89	67	38	75	94
32	20				37, 83	44, 33	53, 43		75	33, 64	80	10, 67	0	41
30	28, 29, 0	0	78, 20, 40, 40		100	89, 25, 89, 89		52	0	72	60	14	94, 100, 75	
28	13, 20		67, 7, 22	0	29, 0		63	40					86, 40	
26	19		0			100	67	67		60			0, 60	
24	0, 44				0									
22	0	7	0			20			100					
20			0											

$T_M.$

Per cent. positive.   Tests beyond the ordinates all negative.

Table XIV where the temperatures are grouped into three classes and the S.R. for each divided into three parts containing approximately equal numbers of tests. The positives, particularly for the lowest T_M interval, are concentrated more in the days with higher maxima.

Incidentally, this suggestion of a greater sensitivity to insolation at lower air temperatures is implicit in Holdaway's findings (1933) that with moderate temperatures *L. sericata* showed a differential haunt preference to *L. caesar*, L., being relatively more numerous in sunny trap sites ; at higher temperatures the difference in frequency of the two species was not related to insolation. The author tentatively interprets this as a negative response of *L. caesar* to insolation, but the alternative explanation of a positive response of *L. sericata* at low temperatures also applies.

Oviposition and Moisture Indices.

The variance of results due to the different moisture indices was in no case significantly greater than the error variance, although there is a considerable discrepancy between treatment and error mean square for the S.D. index (Table X). The error estimates here show disturbance by omitted factors, but this is as likely to be due to the already demonstrated temperature associations having differential effects with different classifications as to the omitted moisture factors.

TABLE X.

Association of oviposition and moisture indices.

Index	Tests	Mean Squares.				P.	Correlation	
		Treatment	Df.	Error	Df.		r.	P.
S.D.	123	142·3	19	97·7	95	>5%	·30	<1%
P	110	107	14	91·5	108	>5%	—·29	>5%
E	115	102	11	89·4	98	>5%	·25	<1%
P/E	110	77·1	20	93·7	89	>5%	—·26	<1%

Some degree of association might be expected for daily S.D., P, and E, since P and E are known to be correlated to some extent with temperature, and S.D. is from its nature obviously so related. Three of the indices do, in fact, show recognisable association trends in the correlation tables. Fig. 2 illustrates the regressions, smoothed in running means of three ; the number of tests, and the number of sheep involved, are shown in the margins. The correlations, calculated from the tables for analysis of variance, are included in Table X. They give low but significant values for S.D., E and P/E, the P index being just over 5 per cent. The difference between the significance estimates by the two methods is probably due to the fact that the analysis is independent of the dispersion of the meteorological index, apart from the grouping attached to it, whereas the correlation test takes into account the covariance of oviposition and the index in relation to their total dispersions.

From the absence of association by analysis of variance test, and from inspection of the regressions, it is clear that the lower values are not due to non-linearity of the association, but to the association being obscured by independent fluctuations of one or other omitted factor, or to the related variation of a factor whose correlation with oviposition is opposite in sign (Table VII, row 4).

The direct inhibitory effect of rainfall on fly activity will obviously not be reflected to any great extent in the negative correlation of daily P and oviposition, since there are no degrees of oviposition during actual rainfall, and since the P index bears little relation to the duration of rainfall. In the months here considered, June to October, the average daily duration, per rain day, lies between 2·1 and 3·7 hours (Bilham 1938, London and Aberdeen data). The effects of daily P must, therefore, contain an indirect component, relating associated or after-effects of rain, whether mechanical or meteorological, to the amount of fly activity during the remaining available hours.

The relatively high correlation for oviposition and log. P/E is interesting in view of the complete absence of association in the analyses of variance. There was no correlation in 1937 ; 1938 and 1939 gave correlation coefficients of −·36 and −·55, both being beyond the 1 in 20 level of significance.
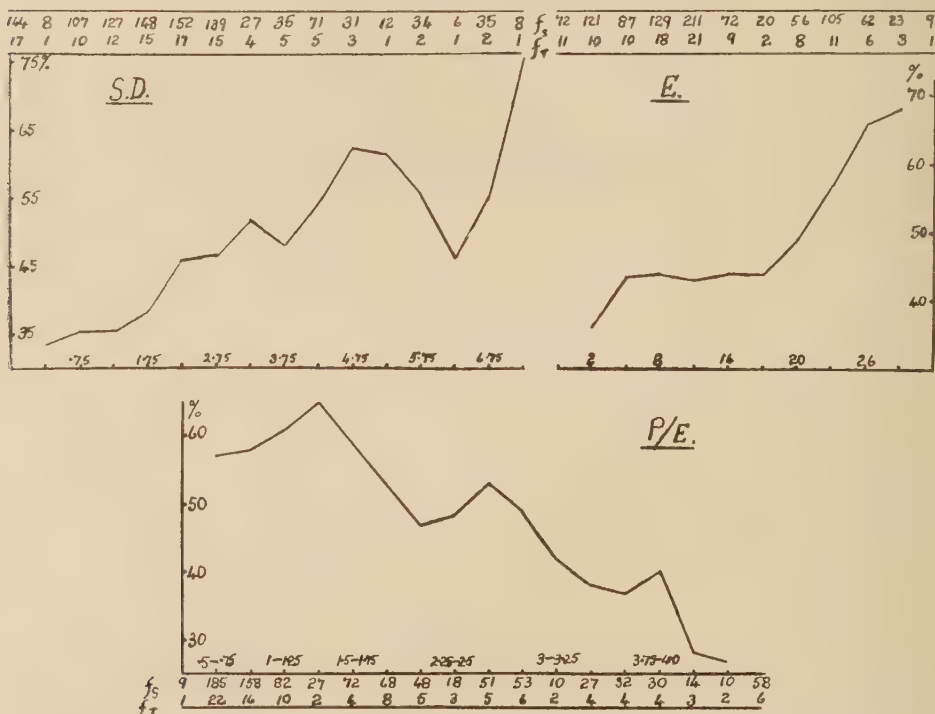
Fig. 2.—Showing the regression of oviposition rate on S.D., E, and P/E (log. $10^4 \times P/E$). The number of sheep (f_s), and of tests, (f_t), at each interval are given in the margin. Scattered tests beyond the upper limits of the S.D. and P/E scales :—S.D., 4 tests and 44 sheep ; P/E, 3 tests and 15 sheep.

Temperature and Moisture Intereffects.

Temperature and saturation deficit. As the saturation deficit was calculated from the daily T_A, this index of temperature was used for examining intereffect. The proportion of oviposition at the higher, medium and lower S.D.'s for different temperature ranges from 9° upwards, is shown in Table XI. The proportion of positives is higher in all cases for high than for low S.D.'s. The partial associations of the sheep ratios with S.D. in the first and third temperature class are consistent in trend and significant by χ^2 tests ; in the middle temperature class the "low S.D." result is aberrant. The observed total S.D. association (fig. 2), although not

detected by the analysis of variance, may thus be a true effect ; further, so far as can be judged from the coarse temperature grading of the table, it appears to be due, to some extent, to a true partial association with S.D., *i.e.* corresponds to row 3 rather than row 1 of Table VII.

TABLE XI.

Saturation deficit × temperature intereffect.

S.D.	Ratio	9°–12°	13°–15°	16°+
High	Days	10 : 12	11 : 12	10 : 10
	Sheep	57 : 107	89 : 125	82 : 127
Medium	Days	9 : 11	9 : 12	9 : 11
	Sheep	24 : 94	43 : 107	64 : 108
Low	Days	7 : 13	11 : 14	7 : 9
	Sheep	25 : 123	97 : 167	44 : 91

Precipitation and temperature. When the results in the different T_M intervals are classified for amount of rain, an interesting result is obtained. For different values of one factor, the response to the other changes in type. This reciprocal interaction effect is illustrated in Table XII. The regression on P has a less steep slope at 18° than at 15-17° ; at 19-21° the regression is practically level for the two lower values, and at temperatures over 21 it is reversed. Similarly, for fixed P intervals, the temperature response changes from a modal curve for no rain to a linear association at high P. The observed curve for either index will, therefore, represent a distortion, due to interaction (MacLeod in press), to the extent to which P and T_M are correlated.

TABLE XII.

Precipitation × temperature interaction.

1 mm. +	8 (48)	28 (72)	44 (73)	53 (34)	70 (59)
< 1 mm.	14 (44)	47 (32)	53 (72)	68 (65)	44 (23)
0	16 (118)	68 (113)	61 (84)	70 (119)	56 (223)
	to 14°	15–17°	18°	19–21°	22°+

Per cent. sheep positive. Figs. in brackets= No. of sheep.

A similar table for S.R. and P gave erratic results and disclosed no consistent trends suggesting an intereffect of P on the response to insolation.

Temperature and the P/E ratio. The excessively biassed distribution of P, with the greater proportion of days in the zero class, limits severely the range of sub-squares which can be compared. The P/E index was, therefore, examined for evidence of the above interaction effect (Table XIII). There is no clear evidence of a true association with P/E, though the rate tends to get higher with low P/E, and there is a suggestion that this trend falls off with rising temperature categories. On the other hand, the effect of given P/E on response to changes of temperature is quite marked. There is obvious interaction, the optimum temperature being lower for the lowest P/E category than for the others. In the T_A table there is an almost diagonal shift of optimum temperature (the rate for the second P/E category, at 13–14°, is not far short of the optimum).

TABLE XIII.

P/E × temperature interaction.

Log. (P/E × 10⁴)		to ·75	·76–1·50	1·51–3·0	3·04+
	−16°	—	32 (78)	30 (37)	16 (55)
	17–18°	63 (60)	66 (71)	49 (65)	39 (94)
T$_M$	19–20°	84 (56)	56 (36)	63 (40)	53 (30)
	21–22°	47 (19)	64 (39)	69 (49)	} 65 (17)
	23°+	34 (59)	95 (43)	56 (119)	
	−10°	—	21 (33)	33 (18)	0 (10)
	11–12°	86 (29)	49 (62)	0 (8)	28 (46)
T$_A$	13–14°	73 (37)	68 (63)	50 (101)	37 (57)
	15–16°	45 (33)	64 (14)	70 (36)	49 }50 (61)
	17°+	50 (95)	73 (95)	60 (147)	53 } (17)

Per cent. of sheep positive. Figure in brackets—number of sheep.

Rain and the temperature × air-drying-power intereffect. A modified two-way table, *i.e.* with relative instead of absolute subclasses of one ordinate, was constructed for T$_M$ and E. In each subsquare the results were further divided according to whether or not rain fell on the day of test. The overall results (Table XIV), might suggest a positive association with E. When, however, rain days are excluded, the days with higher daily totals of evaporation are seen to be no more conducive to oviposition than those with lower daily E—the results rather suggest the reverse, but the inconsistent values for "medium E" confuse the trends for both total and rainless days. No such effect of rain on the S.R. × T$_M$ intereffect is evident.

The results are inadequate to examine in more detail the effect of rain on the temperature × evaporation and temperature × insolation intereffects.

TABLE XIV.

Effect of rain on the air-drying-power × temperature, and insolation × temperature intereffects.

Evaporation	Subclass	Maximum Temperature		
		to 16°	17–19°	20°+
High	Rainless days ..	(6) 13/39 33%	(9) 47/73 64%	(12) 62/101 61%
	Days of rain ...	(2) 5/11 45%		(7) 59/73 81%
	Total	36%	64%	70%
Medium	Rainless days ..	(4) 6/24 25%	(8) 49/79 62%	(5) 33/61 54%
	Days of rain ...	(7) 3/38 8%	(7) 31/76 41%	(5) 17/33 52%
	Total	15%	52%	53%
Low	Rainless days ..	(1) 4/5	(4) 27/32 84%	(8) 90/132 68%
	Days of rain ...	(7) 8/33 24%	(11) 48/115 42%	(4) 19/34 56%
	Total	32%	51%	66%

Insolation		12–14°	15–19°	20°+
High	Rainless days...	(6) 14/66 21%	(13) 82/127 65%	(9) 94/116 81%
	Days of rain ...	(4) 8/46 18%	(4) 19/47 40%	(4) 34/45 75%
	Total	20%	58%	80%
Medium	Rainless days ..	(7) 16/78 21%	(8) 46/73 63%	(7) 40/77 52%
	Days of rain ...	(2) 2/13 15%	(11) 74/145 51%	(5) 22/46 48%
	Total	2)%	55%	50%
Low	Rainless days ..	(7) 1/60	(7) 28/60 47%	(9) 47/97 48%
	Days of rain ...	(4) 0/18	(11) 23/78 29%	(5) 24/35 69%
	Total	<2%	37%	54%

Figures in brackets = the number of days. Ratio = Positive to total sheep.

Review of Meteorological Relations.

The scattered conclusions and suggestions extracted from the foregoing analyses may now be brought together to form a picture of one aspect of the climatology of myiasis, namely, the effect of weather on response of blowflies to a given attractant odour.

There is a definite association of oviposition rate with the T_M index, at max. temperatures between 13 and 26°. This shows in the total association, and is present when the effect of S.R., S.D., E, P, and P/E indices are separately removed. The fundamental association of temperature and response to attractant odour is thus reflected in an association of the daily total of oviposition with one at least of the daily indices of air-temperature. There is also a definite crude, $i.e.$, total, association of oviposition with maximum daily insolation, as measured by the difference between daily maxima for sun and air temperatures.

Theoretical grounds for accepting the association of the S.R. index as a partial, $i.e.$ a true, relation, possibly non-linear, are given in the section on the oviposition $\times$ S.R. association. The existence of a true association is indicated in Table IX although on account of the irregularity of the data it is not conclusively shown. Further, the total association remains unaffected by elimination of rain effects (Table XIV). It is suggested that the association is in fact a true relation, and that consequently there will be a main effect of S.R. and T_M, $i.e.$ that the level of response to T_M will vary with the S.R. index.

The three single indices of moisture, S.D., E, and P, and the ratio P/E, all show some suggestion of association, P and P/E being negatively related to oviposition. The association is in all cases of low degree, and not reliably demonstrable statistically. The apparently low degree of association could be due to positively related variation of another factor whose correlation with oviposition was opposite in sign ; this relationship is, however, not applicable to any pair among the meteorological indices considered, and the low associations more probably reflect the degree of freedom of fluctuation of the indices, relative to the oviposition variable.

The S.D. index, although much of its association will obviously be traceable to the temperature effect, has apparently a degree of partial association, independent of temperature (Table XI). The S.D., therefore, or even the air temperature, could account for the E association. Table XIV suggests that it is a genuine effect ; P and E are not positively correlated, yet when the P factor is eliminated the positive E association disappears, the trend being if anything in the opposite direction. The effect thus seems to be limited to rain days, and may be due to a negative correlation of E and duration of rainfall. Precipitation will obviously have some degree of negative association with oviposition, for mechanical reasons, in addition to a probable illusory component from its known negative association with temperature, and in fact a true P association may be deduced from the evidence, since the P factor can affect the response to T_M.

With regard to air humidity in general, the evidence is very inconclusive. As stated above, E, freed of P and T effects, is if anything negative ; S.D., freed of T, is positive, but this index is much more uncertainly related to daily humidity conditions than is E.

There appears to be a definite interaction of the P factor on the oviposition response to temperature ; with conditions of low P the optimum temperature for oviposition appears to be lower than with high P., $i.e.$ the quality of the response to temperature varies, and not merely the level, as occurred with a gradient of S.R. conditions. The P index is, however, more in the nature of a qualitative indication of an occurrence—rain—than a quantitative measure of a condition affecting insect activities ; it does not indicate the extent of rain-free time left per day. The effect of the P factor on the response of flies to temperature must be indirect, expressing

itself through some condition which is produced or varied by precipitation. The duration of rain is one such condition, amount of distributed free moisture another. The latter affects the humidity level at which aqueous tension will effect a balance with air-drying forces. The P/E ratio is to some extent a measure of either of these, and for interpretation of moisture effects in the ecology of aerial or subaerial insects it would seem preferable to use the ratio rather than the direct measure of precipitation.

The P/E index shows the interaction of moisture on the T_M or T_A response. It is more evident than with P, from the greater number of intervals permitted by the P/E distribution. The reciprocal interaction of T_M on P/E, though suggested in the tabulation, is not clearly demonstrated. The interaction, however, is not a spurious effect of related variation of T_M and P/E (e.g. Table VII, row 8 or 10), since the change of regression can be traced over the same T_M range.

This change of optimum T_M under different P/E conditions means that the regression curve of daily oviposition on daily T_M will not be a fixed curve. In so far, however, as test conditions during the 5 years 1936 to 1940 can be taken as a representative sample of the summer incidence and range of the P/E ratio, the regression of oviposition on T_M, for standard stimulus, should tend to level out for temperatures over 20°, i.e. the fundamental temperature relation is unlikely to be evident in the association with air temperature maxima over 20°, or with daily average temperatures above a probable limit of 12° to 14°.

Hypothesis of weather effect. Consider first the significance of the moisture × temperature interaction effect. Using the direct index P, one must regard precipitation as an interfering factor, modifying the true response obtained under uncomplicated precipitation-free conditions. That is, the true modal type regression curve obtained in absence of precipitation is distorted under impress of precipitation effects into an apparently linear regression. Since the actual regression is the other extreme, i.e. exponential, this interpretation seems improbable. The converse interpretation is that the linear regression obtained with the higher P values is distorted into a modal curve by interference due to absence of P. This interpretation at first glance seems absurd, but makes sense if we replace P with P/E : a low or nil replenishment of free moisture, under conditions of high or moderate dissipation (i.e. a low P/E value) will produce more rapid desiccation effects, i.e. will interfere with the increase of response to oviposition stimulus under conditions of increasing temperature.

If one accepts the lesser hypotheses that the T_M, S.R. and P indices are associated with daily oviposition rate, that the other indices have only illusory or negligible association, and that the P/E ratio of indices affects the nature of the T_M association, the findings may now be linked up to form a pattern of weather effect on oviposition. The general hypothesis is that the oviposition rate per day, for standard stimulus, increases with increasing T_M above a lower critical limit in the neighbourhood of 12–14°. This holds for temperature maxima up to at least 26°. The rate of increase for the higher levels of temperature is, however, modified by a variable moisture effect, which is expressible by the P/E ratio. It is suggested that this moisture effect is desiccation. Its operation results in a falling off of oviposition rate when a given temperature is surpassed ; this optimum T_M value is higher for high values of daily P/E and drops to the neighbourhood of 20° when the balance of available moisture and air-drying-power, as measured by P/E, is low. For given conditions of air temperature, insolation steps up the response to an attractant stimulus.

The preliminary analysis of year effect on the results showed that there was a significant difference in level, and suggestive difference in quality, of oviposition response between years. The consequences of the above hypothesis may therefore be tested by analysing the phenomena for 1937. Climatically the year was abnormal. Reference to the annual summary of the Meteorological Office Monthly Weather

Report (1937), shows that the year was characterised over most of the country by very dry or absolute drought conditions from June to November, and a marked deficiency of sunshine for much of the year. The distribution of observed S.R. and P/E during the fly months was aberrant. The proportion of test days with low S.R. is greater than the mean over all years. Thus, as compared with the all-years total, the 1937 percentage of test days with S.R. maxima of under 25°, 26–32°, and over 32°, is as follows :—

<div style="text-align:center">

All years (total days 125)   19%   50%   31%
1937 (,,   ,,   51)   29%   55%   16%

</div>

Inspection of Table VI shows that the number of sheep tests under low P/E conditions (99), is relatively greater in 1937 than in any other year.

These being the conditions, the mean oviposition rate in 1937 for given temperatures should, according to the hypothesis, be reduced and the optimum temperature of the mean regression should be lower, as compared with the results over all years.

The 1937 mean oviposition rate, as determined by fitting regression constants, *i.e.* comparative to other years in respect of T_M, was lower than that for any of the other four years (see footnote, p. 186). Analysis of the year effect showed that there was a suggestive difference in the shape of the oviposition regression on T_M and S.R. for 1937, though the difference was not significant in the analysis of the variance. In the section on the regression of air-temperature, the 1937 curve is examined in another manner, and appears to differ significantly from the others. It has an apparent optimum at 18 to 22°.

Discussion.

The effect of meteorological factors on insect activity is, even in its most simplified form, a complicated problem. The factors are related among themselves ; the insect population is subject to change from causes other than meteorological ; and phenological effects may weight the results. Many of the technical and philosophical questions confronting the student of data on such problems are discussed in detail by Williams (1940).

In the present work it has been possible to avoid some of the confusion by first examining directly the effect of certain meteorological factors. This examination, reported in part I, showed that oviposition was principally governed by temperature, and that relationships observable with humidity and insolation were for the greater part illusory. The assumption that a temperature relationship does in fact exist has been basic to the studies reported here, which have concentrated on examining (*a*) the extent to which daily temperature indices reveal this relationship, (*b*) the extent to which divagations can be related to changes in other indices, and (*c*) the reliability of observed associations with other indices, whether genuine or spurious in origin, as measures of oviposition in relation to weather.

Three interesting discrepancies have been revealed between the relation with the actual factors and that with the daily indices of these factors.

(*a*) Oviposition has a probable but not conclusive negative association with insolation ; there is, however, a quite definite positive association with the insolation index. No explanation of this is attempted.

(*b*) Air temperature is linearly related to log. oviposition, up to 22°C. (actual), but appears to have a quite different regression curve when measured by daily max. temperature. The relationship would seem to be obscured by factors not operative under the critical tests, and the hypothesis is advanced that the interfering factor is the available moisture, expressible as P/E.

(*c*) Daily indices of air moisture, expressed as saturation deficit and as evaporating power, show a negative association of oviposition and humidity.

When, however, the effects of actual precipitation are eliminated, the E relation disappears. To the extent that E is a function of humidity, oviposition is thus not less with higher humidity, on rain-free days, when judged by daily values of both.

When the effect of rain is eliminated from the insolation effect, the positive correlation with insolation still holds, dry sunny is more favourable than dry cloudy weather. The practical farmer maintains that oviposition is favoured by sunshine and high humidity. On the other hand, the more critical study of isolated effects appeared to disprove the contention, and in the present study the humidity, as judged by the estimated mean S.D., is inversely related, even apart from the associated temperature effect. Admitting the unreliability of this last index, the question of the relation of humidity to oviposition must still be left open.

The distribution of tests does not allow a full comparison of seasons, *i.e.* of cooler early and late months against the hot middle season months. Comparison of summer, mainly July and August, and autumn, September and October, results failed to reveal a significant season effect, but, on the evidence, the possibility cannot be regarded as dismissed.

Comparison of the oviposition results for different years revealed that 1937 was aberrant. A possible explanation of this is brought out in the text, in relation to the hypothesis of weather effect, but some general aspects may be touched on here.

The level of the oviposition response was lower in 1937 than in other years. The explanation of this might be sought in either climatic or biological causes, *e.g.*, in a lowered population density. It is, however, simply explicable as an effect of the relatively reduced insolation for that year. There is also an apparent difference in the kind of response to temperature. The change from positive to negative in the response to temperature increase in 1937 would show itself in statistical analysis in the discrepancy between the expected and the actual sum total of the variance due to the temperature and year factors. That the amount of this discrepancy—the interaction variance—is not significant does not mean that the suggestion of a different type of response in 1937 is false. The significance of interaction is calculated from the ratio of the interaction variance to the basic variance of individual results ; the dispersion of results in this body of data is such that the variance is in the order of 8,000, *i.e.*, a standard deviation of $\pm$ 28 about a calculated mean per cent. With such dispersion only gross effects can pass the statistical test of significance.

If it is accepted that the interaction effect of year and temperature does exist, and is due to 1937 aberration, it cannot be attributed to biological causes, as is possible with the previous effect—the change in oviposition level. A change of sheep breed might conceivably result in an altered optimum temperature for operation of the attractant stimulus, but there was no essential difference between 1937 and the other years in the variety of breeds used. The cause must, therefore, be entirely climatic, and, on the basis of the hypothesis of weather effect on oviposition put forward in this paper, the change of regression type is in fact explicable in terms of climatic conditions for the year in question.

Summary.

Records of oviposition by blowflies on test groups of sheep, over a five-year period, were examined for relation between the rate of oviposition per day and the conventional meteorological indices of daily weather conditions. There was a definite correspondence with the maximum shade temperature for the day. The relation was not straight-line, the slope changing at between 18 and 20°C. The association with the minimum temperature was not significant. Insolation, as measured by the difference between maximum temperatures in the sun and shade, was positively associated with oviposition.

The humidity indices—evaporating power of the air and the saturation deficit—and the ratio of the daily totals of rain and evaporation, showed a low correlation ; the rainfall correlation was barely significant. The association was in no instance statistically reliable.

For given temperatures, higher insolation was associated with greater oviposition. This intereffect was not affected by the rain factor. High saturation deficit and high evaporation were similarly positively associated with oviposition, but the evaporation effect was found to be true only for days when rain fell. Variation of the P index or of the P/E ratio appeared to result in temperature having differential effects ; the optimum temperature was apparently lower with the lowest P or P/E value than with higher categories.

The hypothesis is tentatively offered that oviposition, for standard conditions of stimulus, is primarily associated with temperature, the regression of daily total oviposition on the maximum temperature being approximately linear between 13° and 26°C. Desiccating effects normally cause curvature of the regression at temperatures above 20°, the optimum maximum temperature becoming less with decreasing available moisture, expressible as daily P/E. For given air-temperature and moisture conditions, the level of response is positively associated with intensity of insolation as measured by the difference of daily maxima of sun and air temperatures.

Acknowledgements.

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THE BIOLOGY OF *QUETTANIA COERULEIPENNIS* SCHWARZER (COLEOPTERA) IN BALUCHISTAN.

By Nazeer Ahmed JANJUA, M.Sc. (Hons.), F.R.E.S., and Ram Narian MEHRA, M.Sc.

Department of Agriculture, Baluchistan.

The Cerambycid, *Quettania coeruleipennis*, Schwarzer, is relatively common in Baluchistan and in recent years damage by its larvae to fruit trees has increased to such an extent that it has now become a pest of major importance. A brief account of the biology was given by Janjua and Samuel (1941). In view of its importance, the authors studied the biology of the insect between 1943 and 1946 with the following results.

Distribution.

The genus *Quettania*, which is so far represented by one species, *Q. coeruleipennis*, Schwarzer, at present occurs only in Baluchistan (Pakistan). It was first recorded by Gardner (1927) from apricot stems received from Quetta and subsequently by Janjua and Samuel (1941) attacking cherry trees at Quetta. As a result of an extensive survey carried out by the authors during the past four years, it has been found to be relatively common in the districts of Quetta-Pishin and Loralai where it occurs at an elevation of from 4,500 to 6,000 feet. The attack has been observed to be more severe at Quetta (5,500 ft.) and Loralai (4,700 ft.) and it is gradually spreading to other parts of the province.

Nature and Extent of Damage.

The larvae of this species of the tribe Callichromini attack the top shoots and small branches of fruit trees. On hatching, the young larva starts boring from one side into the shoot or branch on which the egg has been laid. After making a small hole which is marked by a gum globule, it works its way into the core of the shoot and tunnels down. As the larvae work their way downwards, they leave a watery fluid which oozes out of the occasional holes made by the larvae to the sides of the branches, thus enabling active damage to be readily detected. The attacked portions of the shoots and branches ultimately become hollow and dry up, the dried leaves remaining attached to such shoots even during the winter season. The attacked shoots and branches also fall off with moderately strong winds. Old trees are the most susceptible. It has been estimated that 10–15 per cent. of the fruit trees are at present infested by this pest but it is gradually spreading.

Food Plants.

The larvae have previously been recorded attacking apricot (Gardner, 1927) and cherry (Janjua & Samuel, 1941). The authors have also found it seriously infesting almond and pomegranate of which it is now a pest of major importance. Other trees attacked are plum and quince.

Description.

Egg.—The egg is laid as a plastic mass which adapts its shape to the place where it is deposited. The majority are laid on the branches where they appear as elongate convex bodies with widely flattened bases more or less resembling newly born *Cypraea* (cowries). The egg is covered on the exposed surface with an opaque whitish cement-like substance which hardens after oviposition, the dark yellow colour

of the contents being still visible.   The flattened areas are covered with a fine trans-
parent membrane only.   They are always laid singly and on the average measure
2·1 mm. long, 1·2 mm. wide at the broadest place and 0·9 mm. high at the highest
point.

The egg does not crack or split ; the larva hatches from the underside leaving the
shape of the shell intact.

Larva.—The newly hatched larva is about 3 mm. in length ; bright yellow in
colour with castaneous mandibles and one distinct ocellus on each side of the head.
Accessory joint of antenna nearly as long as true terminal joint.   Lingula of labium
extremely small.   Pronotum striate posteriorly and with a distinct median line.
Abdominal ampullae more or less bilobed, minutely granulate.   Spiracles bilabiate ;
the mesothoracic spiracles widely oval or subcircular, appearing on the surface as a
cup-shaped depression at the base of which is an oval aperture provided with 14 or
15 finely branched peripheral processes ; abdominal spiracles circular.

Immediately on hatching, the larva wriggles about and starts boring into the
shoots at a distance of about 8 mm. from the place where the egg has been laid. After
reaching the core, the larva takes a downward course until it becomes full grown
and has caused the damage described above.

The full grown larva is about 4 cm. in length and about 5 mm. in breadth, dirty
creamy white in colour with brownish head carrying short strong mandibles.   A
detailed description of the mature larva has been given by Gardner (1927).

Pupa.—When about to pupate, the larva constructs a wide chamber made at the
end of the tunnel which is closed on all sides by sawdust packing.   The pupa has the
form of the adult except that its parts are soft and is of creamy-white colour.   Its
length is about 3 cm. and breadth about 6 mm.   The antennae are long, recurved
near the 7th abdominal segment, the apical joint reaching near the head in the male.
Pronotum with a number of soft erect papillae, each papilla with a short cephalad
seta.   Abdominal tergites 1 to 6 without papillae or setae ; 7th tergite with a
posterior group of papillae similar to those on the pronotum but smaller ; 8th tergite
with a straight median posterior projection and with a few papillae.

Adult.—Schwarzer (1931) described the adult beetle.   When about to emerge,
the adults cut holes directly to the outside from which they crawl out.   They are
slender, bronze in colour with green reflections, sun loving insects and are seen sitting
on branches and twigs on bright sunny days.

Duration of various Stages and seasonal History.

A preliminary account of the seasonal history has been given by Janjua and
Samuel (1941).   As a result of investigations carried out by the authors it has been
ascertained that the beetle takes one year to complete its life-cycle.   The studies
were started with eggs deposited in 1943.

Observations during the years 1943–1946 showed that oviposition commenced
between 7th and 18th May and ended between 22nd May and 3rd June.   The
incubation period varied from 12 to 19 days with an average of 15·9 days. Hatching
commenced between 22nd May and 2nd June and ended between 10th and 20th June.

The larva on hatching feeds on the soft sappy bark until its mouth-parts are
strong enough to attack harder substances when it burrows into the branch.   As it
grows in size, the tunnel is also enlarged.   The larva does not allow the sawdust to
accumulate in the tunnel ; it is expelled through the occasional holes made in the
sides.   When the tunnel is cleansed of sawdust, these holes are plugged by the larva.
The watery fluid left by the larva usually oozes out of these plugged holes. It has been
observed that a larva can bore a tunnel about three feet long and $\frac{1}{2}$ inch wide within
which it can freely turn round.   The summer and winter months are spent by the

larvae in making these tunnels where they remain thereby escaping cold. As spring approaches the larva enlarges the tunnel at the bottom and makes the pupal chamber. The feeding period of the larvae ranged between 265 to 317 days with an average of 289·6 days.

The pupal chamber formed by the larva is straight, about 5 cm. in length. When full fed, the larva plugs the top with gnawed wood and constructs a thick wall of some calcareous substance, lining the sides of the chamber with a film of fibrous material. The prepupation period varied from 7 to 12 days with an average of 9·7 days.

During the four years, pupation first took place between 2nd and 10th March and the last between 12th and 24th March. The pupal period varied from 25 to 36 days with an average of 30·2 days.

When transformation is complete, the adult, as soon as its outer parts have hardened, bores its way out of the tree by gnawing a hole straight to the outside through the wood. The beetle does not, however, always leave the tree in this manner. It often, perhaps usually, simply breaks the calcareous partition made by the larva at the top of the pupal chamber and crawls up the tunnel and gnaws its way through one of the holes made by the larva after removing the plug. Emergence of the adults began between 5th and 15th May and ended between 18th and 29th May.

The beetles pair soon after emergence, copulation, lasting for about 20 minutes, takes place in the morning hours. The pre-oviposition period varied from 2 to 7 days with an average of 5·3 days.

The average number of eggs deposited per female was 59·7—ten females depositing 597 eggs. The maximum number of eggs deposited by a single female was 74.

The life-history data are summarised below :—

Year	First eggs deposited	Oviposition ended	Incubation (days) Range-Average	Hatching of eggs From	To	Feeding period of larvae (days) Range-Average
1943 ...	15.v	30.v	13–17	29.v	16.vi	265–298
1944 ...	18.v	3.vi	15–18 (15.9)	2.vi	20.vi	275–290 (289·6)
1945 ...	7.v	22.v	15–19	22.v	10.vi	295–301
1946 ...	12.v	27.v	12–18	25·v	15.vi	290–317

Year	Prepupal period (days) Range-Average	Pupation From	To	Pupal period (days) Range-Average	Emergence of adults From	To	Life-cycle (days) Range-Average
1944	7–9	2.iv	12.iv	29–35	15.v	29.v	354–379
1945	8–10 (9·7)	8.iv	19.iv	25–30 (30.2)	5.v	18.v	343–368 (362·9)
1946	7–11	4.iv	15.iv	30–36	14.v	21.v	357–380
1947	8–12	10.iv	24.iv	27–32	10.v	24.v	347–374

(The above observations are based on 30 individuals.)

Summary.

The biology of the Cerambycid, *Quettania coeruleipennis*, which is a serious pest of almond and pomegranate in Baluchistan is described. The larvae attack the top shoots and small branches, tunnelling downwards ; the attacked branches ultimately become hollow and dry up.

The various stages are described in detail and their duration and seasonal history during the years 1943–1946 have been investigated. During these years, oviposition

commenced between 7th and 18th May and ended between 22nd May and 3rd June; the incubation period of the eggs ranged from 12 to 19 days (average 15·9 days). Hatching of eggs started between 22nd May and 2nd June and ended between 10th and 20th June and the feeding period of the larvae ranged from 265 to 317 days (average 289·6 days). The prepupal period varied from 7 to 12 days (average 9·7 days). The first pupation during these four years took place between 2nd and 10th March and the last between 12th and 24th March and the pupal period varied from 25 to 36 days (average 30·2 days). Emergence of the adults began between 5th and 15th May and ended between 18th and 29th May.

The beetles pair soon after emergence and the pre-oviposition period varied from 2 to 7 days (average 5·3 days). The average number of eggs deposited per female was 59·7 and the maximum number of eggs laid by a single female was 74. The life-cycle ranged from 343–380 days with an average of 362·9 days, the beetle thus takes one year to complete its life-cycle.

Acknowledgements.

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AN ARSENIC-RESISTANT TICK AND ITS CONTROL WITH "GAMMEXANE" DIPS.—PART II.

By A. B. M. WHITNALL, M.Sc. and B. BRADFORD, M.Sc.

CONTENTS.

Introduction.

In part I of this study (Whitnall & Bradford, 1947) it was shown that an arsenic-resistant strain of the one host blue tick, *Boophilus decoloratus*, Koch, existed in South Africa. Originally the tick occupied a localised area around East London but it spread rapidly, and by 1945 it had established itself northwards in Zululand, while

Fig. 1.—The neck of a cow infested with arsenic-resistant ticks. Such infestations were a common sight in the Bathurst area in spite of regular weekly dippings in 0·16% As_2O_3.

to the south it had spread along the coastal areas to the Alexandria district of the
Eastern Cape. The arsenic resistance seemed to be inherited, but certain climatic
conditions appeared necessary for the resistance to manifest itself, or for this strain
of tick to survive within the natural habitat of the blue tick. Thus, if cattle infested
with arsenic-resistant ticks were transferred from the Bathurst area on the coast
to the Fish River area further inland, the ticks disappeared, being either unable to
survive in the new environment, or succumbing to normal arsenical dippings. If this
is the case, then the menace to the cattle industry is not so serious as was at first
anticipated. Nevertheless as Thorburn (1947) has shown, the present distribution
of the arsenic-resistant tick seriously affects a large and important cattle ranching
area of South Africa. In the Bathurst area, where the present study was conducted,
severe drought in 1945 and 1946 coupled with heavy tick infestations (fig. 1),
resulted in the death of many cattle despite regular weekly treatment in a dip
containing 0·16 per cent. As_2O_3.

In part I particular attention was given to a laboratory technique for the *in
vitro* treatment of ticks, and it was shown that gamma isomer of hexachlorocyclo-
hexane was very toxic to adult female arsenic-resistant ticks. The work to be
discussed concerns laboratory experiments with preparations likely to be suitable
for use as cattle dips together with the results of extensive field experiments conducted
between March, 1946 and April, 1947.

Laboratory Experiments.

Hexachlorocyclohexane is known to have at least four isomers, alpha, beta,
gamma and delta and its insecticidal properties are mainly attributable to the gamma
isomer, "Gammexane". When the work described in this paper was first com-
menced, the crude hexachlorocyclohexane was thought to contain about 10 per cent.
of the gamma isomer, but with improvements in analytical technique, it is now
known that it may contain up to 14 per cent. The figure 10 per cent. has been
taken for purposes of calculation throughout this paper except where otherwise
stated. In all cases the calculations are based on the gamma isomer content of the
original crude hexachlorocyclohexane, and not on that in the made-up formulations.

Many different dip formulations were prepared for biological examination, using
adult female ticks and the *in vitro* technique described in part I. The results are
presented in Table I.

TABLE I.

Formulation No.	No. of ticks	Gamma isomer content of diluted formulation	Per cent. females laid	Per cent. batches hatched	Per cent. control
Water ...	20		100	100	0
P.530 ...	50	0·001%	90	93	16
		0·002%	42	67	72
		0·004%	8	50	96
D.P.40/B ...	50	0·001%	96	96	8
		0·002%	36	78	72
		0·004%	4	0	100

Twenty ticks in water. Fifty ticks in each "Gammexane" treatment.

P.530. Dispersible powder containing 50 per cent. crude hexachlorocyclohexane known to
contain 14 per cent. gamma isomer.

D.P.40/B. Dispersible powder containing only pure gamma isomer.

Altogether 119 separate *in vitro* experiments were carried out, involving the treatment of 20,000 adult female ticks. In this way a biological examination of all formulations was conducted as a check on the estimated gamma isomer content. Samples of dip washes from experimental tanks were also tested biologically. All these tests, some of which will be discussed below, were a vital supplement to chemical analyses, where only the total hexachlorocyclohexane and not the gamma isomer was determined.

Emulsions.

The first samples of formulations that might prove suitable for use as cattle dips, were oil base preparations received in July 1945. An oil phase in water emulsion, the oil being an extract of hexachlorocyclohexane in coal tar oils, appeared best. It was tested at various dilutions and the results of some tests, presented in Table II, indicated that 0·005 per cent. gamma isomer should prove a satisfactory concentration for use in the field.

TABLE II.

Formu-lation No.	Exp. No.	Dilution	Grams gamma isomer in 100 mls. dilution	"Gammexane" Tests				Water Test	
				No. ticks	% ♀♀ laid	% batches hatched	% con-trol	No. ticks	% con-trol
2.	16	1/1000	0·0009	20	25	40	90	60	10
		1/500	0·0018	20	35	29	90		
		1/250	0·0036	20	5	0	100		
	18	1/400	0·0022	20	40	38	85	60	12
		1/300	0·0030	20	30	33	90		
		1/200	0·0045	20	25	20	95		
		1/150	0·0051	20	5	0	100		
		1/100	0·0089	20	10	0	100		
		1/50	0·0178	20	5	0	100		
	79	1/500	0·0018	100	30	77	77	20	25
		1/400	0·0022	100	33	73	76		
		1/300	0·0030	100	10	40	96		
		1/200	0·0045	100	4	0	100		
2A.	16	1/1000	0·0009	20	60	42	75	60	10
		1/500	0·0018	20	45	33	85		
		1/250	0·0036	20	15	0	100		
	18	1/400	0·0022	20	65	46	70	60	12
		1/300	0·0030	20	50	20	90		
		1/200	0·0045	20	30	67	80		
		1/150	0·0051	20	15	33	95		
		1/100	0·0089	20	25	60	85		
		1/50	0·0178	20	25	40	90		

2. Oil in water emulsion, the oil phase being an extract of crude hexachloro-cyclohexane in coal tar oils.

2A. Oil emulsion added to a water solution of sodium arsenite containing 0·16% As_2O_3.

It would also appear from Table II that the insecticidal properties of the emulsion were not increased when it was added to a water solution of ordinary commercial sodium arsenite containing 0·16 per cent. As_2O_3. The keeping qualities of the oil in water emulsion appeared to be sound, so that the first field tests were carried out with it. Other oil base preparations were received in February, 1946, and from these

the most promising were selected by *in vitro* tests. Some results obtained with Formulation No. 27, an oil in water emulsion, the oil phase being an extract of crude hexachlorocyclohexane in a mixture of coal tar and mineral oils, and with Formulation No. 28, a miscible oil containing an extract of crude hexachlorocyclohexane in a mixture of coal tar and mineral oils, are given in Table III.

TABLE III.

Formu-lation No.	Exp. No.	Dilution	Grams gamma isomer in 100 mls. dilution	No. ticks	" Gammexane " Tests			Water Test
					% ♀♀ laid	% batches hatched	% con-trol	% control
27	81	1/400	0·0062	50	6	0	100	10
	87	1/400	0·0062	50	14	57	92	18
	92	1/800	0·0031	20	5	0	100	25
		1/600	0·0046	20	5	0	100	
		1/400	0·0062	20	10	0	100	
28	81	1/1000	0·0067	50	10	0	100	10
	87	1/1000	0·0067	50	4	0	100	18

27. Oil in water emulsion, the oil phase being an extract of crude hexachlorocyclohexane in a mixture of coal tar and mineral oils.

28. Miscible oil containing an extract of crude hexachlorocyclohexane in a mixture of coal tar and mineral oils.

The results in Table III confirm the toxicity of the gamma isomer to adult female blue ticks, and it appeared from the extensive *in vitro* tests that if such formulations were used at dilutions giving 50 p.p.m. "Gammexane", a satisfactory control of the tick in the field could be expected. Formulation No. 2, used at a dilution of 1 in 200 was considered too bulky for extensive field work. Formulation No. 28 used at a dilution of 1 in 1,000 was very satisfactory, but the majority of the field work was carried out with Formulation No. 27, which for practical purposes was diluted at the rate of 1 to 400.

The *in vitro* work with oil base preparations did not always give consistent results against adult female ticks. When these anomalies were examined further, they were found to be due partly to the ratio of oil to " Gammexane " in the emulsion. The effect of varying this ratio is clearly demonstrated in Table IV.

TABLE IV.

Description of formulation	Oil : " Gammexane " ratio	% " Gammexane "	% females laid	% batches hatched	% control
Water			100	100	0
Miscible oil contain-ing pure gamma isomer in solvent naphtha	313 : 1	0·0048	66	70	54
	100 : 1	0·0044	50	36	82
	50 : 1	0·0046	44	50	78
	10 : 1	0·0052	34	0	100

Twenty ticks in water. Fifty ticks in each " Gammexane " treatment.

The figures in column 2 were obtained by diluting a standard miscible oil preparation of pure gamma isomer with further quantities of solvent naphtha. These miscible oils were then diluted with water to give a calculated gamma isomer content of 50 p.p.m. Chemical analyses were carried out on these dilutions, the results of which are tabled in column 3. As the standard miscible oil preparation contained only the pure gamma isomer, it naturally follows that the figures in column 3 are actual results and not calculated.

Suspensions.

Some of the results obtained from a series of experiments with dispersible powders containing " Gammexane " are given in Table V.

TABLE V.

Formu-lation No.	Exp. No.	Dilution Gms./litre	Grams gamma isomer in 100 mls. dilution	No. ticks	"Gammexane" tests			Water test
					% ♀♀ laid	% batches hatched	% con-trol	% control
18	95	0·17	0·0025	20	5	0	100	5
		0·34	0·005	20	0	0	100	
		0·68	0·01	20	5	0	100	
19 C		0·575	0·0025	20	10	0	100	
		1·15	0·005	20	10	0	100	
		2·30	0·01	20	0	0	100	
20 A		0·59	0·0025	20	35	29	90	
		1·18	0·005	20	10	0	100	
		2·36	0·01	20	5	0	100	
22 A		0·415	0·0025	20	30	33	90	
		0·83	0·005	20	5	0	100	
		1·66	0·01	20	0	0	100	
19 C*		1·15	?	20	35	100	65	
1 M	109	2·0065	0·0065	50	16	63	90	0
1 N		2·003	0·0033	50	24	42	90	
1 P		2·0013	0·0013	50	38	63	76	

Nos. 18, 19 C, 20 A, 22 A. Dry powders consisting of a benzene extract of crude hexachloro-cyclohexane deposited on china clay.

No. 19 C.* The supernatant liquor from Formulation 19 C above.

Nos. 1 M, 1 N, 1 P. Dry powders consisting of benzene solution of pure gamma isomer deposited on china clay.

Generally, better results were obtained with formulations based on extracts of crude hexachlorocyclohexane as compared with those based on the pure gamma isomer, and this suggested that the amount of gamma isomer in the former was underestimated.

Further experiments with suspensions compared powder and paste formulations of crude hexachlorocyclohexane, the pure gamma isomer and DDT, and the results obtained from such comparative tests are given in Table VI.

From the table it will be seen that " Gammexane " is more toxic to the adult female blue tick than DDT. Thus DDT at concentrations of up to 0·4 per cent. failed to give 100 per cent. control, while a gamma isomer of hexachlorocyclohexane

of 0·005 per cent. gave, in practically every instance, complete control. Furthermore it was estimated that dips of either the oil emulsion or suspension type, to give this concentration of the gamma isomer on dilution, could readily be manufactured at economical costs. All experiments in the field were thus directed to tests with hexachlorocyclohexane preparations.

TABLE VI.

Formu-lation No.	Exp. No.	Dilution Gms./litre	Grams gamma isomer in 100 mls. dilution	Biological data			
				% ♀♀ laid	% batches hatched	% control	Water % control
1 A	100	2·016	0·005	4	0	100	8
1 B		2·008	0·0025	10	0	100	
1 C		2·004	0·001	28	14	96	
1 D		3·023	0·005	18	0	100	
1 E	102	2·005	0·005	24	8	98	4
1 F		2·003	0·0025	30	33	90	
1 G		2·00	0·001	48	33	84	
1 H		2·5	0·005	26	0	100	
			Gms. p.p. DDT in 100 mls. dilution				
2 A	100	2·5	0·4	54	11	94	8
2 B		2·25	0·2	74	38	72	
2 C		2·1	0·08	90	80	28	
2 F		3·73	0·4	70	23	84	

Fifty ticks in each treatment.

1 A–D. Benzene extract of crude hexachlorocyclohexane deposited on china clay. 1 A, B, C dry powders ; 1 D dispersible paste.
1 E–H. Benzene solution of pure gamma isomer deposited on china clay. 1 E, F, G dry powders ; 1 H dispersible paste.
2 A–C, F. Benzene extract of DDT deposited on china clay. 2 A, B, C dry powders ; 2 F dispersible paste.

Larval ticks and the persistent action of " Gammexane."

Large numbers of larval ticks were bred from controls in the course of experiments with adult females. These larvae, the progeny of the arsenic-resistant strain of *B. decoloratus*, were used to test the toxicity of the gamma isomer to this stage of the tick.

When tubes, containing approximately 4,000 larvae, were completely immersed in a dilution of Formulation No. 2 containing 0·0073 per cent. of the gamma isomer, all the larval ticks were dead after four hours. The controls, treated in water, were still alive four days later, when the experiment was discontinued.

This procedure was varied to overcome the difficulty of draining and drying tubes of larvae. Strips of paper, which had been allowed to dry after treatment in the diluted dip, were inserted into the tubes one hour after being moistened. The results were again striking. Forty minutes after papers, treated in Formulation No. 2 containing 0·0073 per cent. of the gamma isomer, had been inserted into tubes, the larval ticks started dying off and after three hours they were all dead. On the other hand the larvae in the control tubes, which contained strips of paper treated in 0·16 per cent. As_2O_3, 0·16 per cent. As_2O_3+0·04 per cent. nicotine and water, lived much longer.

The same method was followed using papers treated in different dilutions of Formulation No. 27, inserted into the tubes twelve hours after treatment. The effect of the freshly prepared dilutions on the larval ticks is illustrated in fig. 2(a), while the action of the dirty wash from dipping tanks is shown in fig. 2(b). The gamma isomer is soluble in distilled water to the extent of ten parts per million (Slade, 1945); papers treated in a freshly prepared saturated solution had no effect upon larvae.

In other experiments, designed to test the persistent action of "Gammexane", strips of treated papers were introduced into tubes of larvae at varying intervals after treatment. Some of these treated papers were exposed to the weather, while others were kept in the laboratory, thus protected from the effects of the weather. It would appear that under the conditions of this experiment, "Gammexane" has a marked persistent action. Papers protected in the laboratory proved toxic to larval ticks up to seven days after treatment, while the toxicity of papers exposed to the weather may last for only three days.
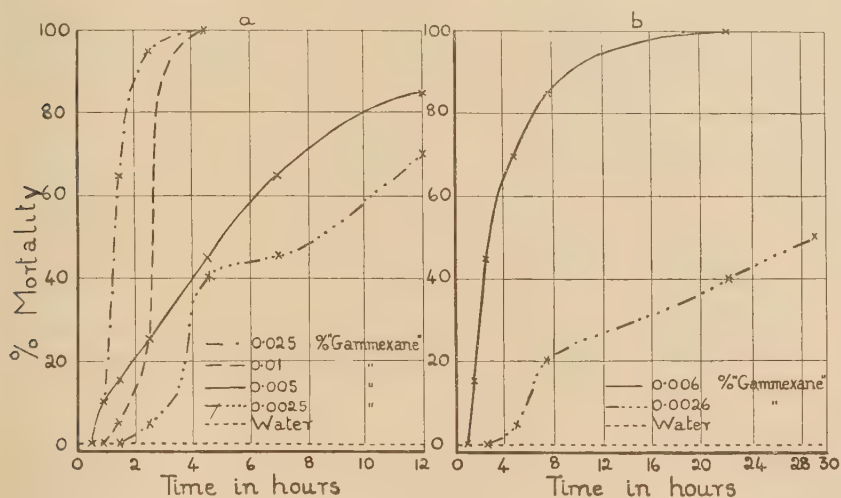
Fig. 2.—Mortality rates of larval ticks subjected to (a) different concentrations of freshly diluted "Gammexane" dip and (b) dirty wash from dipping tanks.   Each graph represents the average of observations on five tubes, each containing about 4,000 larvae.

These laboratory results indicated that excellent control of larval ticks could be anticipated in the field, where the hairy coats of cattle would retain the "Gammexane", the persistent action of which would be operative even when applied to an animated surface.

As will be shown when the field experiments are discussed, weekly dippings kept cattle free from all but the larval stage of the single host blue tick. The presence of larvae on both dipped and undipped animals indicated active blue tick life on the pastures and that "Gammexane" had no repellent action on this stage of the tick. Larvae scraped from undipped animals at weekly intervals were in various stages of engorgement, the average length of fifty being 1·05 mm. Some larvae scraped from the dipped animals were dead when removed and many others were unengorged; the average length of fifty was 0·75 mm. It seemed that only those which attached themselves some days after the animals were dipped were able to feed and grow in size. These observations were taken as further indications of the persistent action of "Gammexane".

Some other common species of ticks and their reactions to " Gammexane ".

Other species of ticks encountered on cattle in the Bathurst and Albany districts included *Rhipicephalus evertsi*, Neumann (the two-host red tick), *R. appendiculatus*, Neumann (the three-host brown tick), *Amblyomma hebraeum*, Koch (the three-host bont tick), *Haemaphysalis silacea*, Robinson (the three-host Fish River Bush tick), *Hyalomma* spp. (the two- or three-host bont legged ticks) and *Ixodes pilosus*, Koch (the three-host sheep paralysis tick). These six species of ticks are as yet readily killed by arsenic.

R. evertsi.

Fully engorged female red ticks responded to *in vitro* treatment with insecticides as follows : Water—0 per cent. control, 0·16 per cent. As$_2$O$_3$—100 per cent. control, Formulation No. 2, diluted to contain 0·0073 per cent. gamma isomer—92 per cent. control.

Owing to the habits of this species, it is necessary to hand-dress under the tails of cattle and in the ears, to effect satisfactory control in the field. Weekly dressings with For lation No. 27, diluted 1 in 250 (0·01 per cent. gamma isomer) kept these parts of cattle commercially free from this tick in large-scale field trials.

R. appendiculatus.

Some *in vitro* experiments were carried out with fully engorged female brown ticks and Table VII records how the ticks responded to treatments in " Gammexane " and arsenic.

TABLE VII.

Treatment	No. ticks	% ♀♀ laid	% batches hatched	% control
Water	20	100	100	0
0·08% As$_2$O$_3$	25	56	21	84
0·16% ,, ...	25	0	0	100
0·002% " Gammexane " ...	25	4	100	96
0·003% ,, ...	25	0	0	100
0·005% ,, ...	25	0	0	100

The formulation used consisted of a dispersible paste containing 50 per cent. crude hexachlorocyclohexane and even 0·002 per cent. of the gamma isomer appeared to be very toxic. The tick was not arsenic-resistant, as 0·08 per cent. As$_2$O$_3$ had a marked effect upon it, while treatment in 0·16 per cent. As$_2$O$_3$ gave 100 per cent. control.

A. hebraeum.

In vitro experiments were not carried out with this tick, but field observations on cattle treated weekly with a spray containing 0·005 per cent. gamma isomer showed that the adult male was very susceptible to this concentration and that it prevented the adult female bont tick from engorging. Large clusters, about four inches in diameter, of adult males and females were successfully dealt with by weekly hand-dressings, using Formulation No. 27 containing 0·01 per cent.

H. silacea.

The life-history of this tick is not fully understood. Larvae, nymphs and adults have been collected from cattle in the Albany District. Adult females treated *in vitro* with Formulation No. 27 containing 0·0062 per cent. gamma isomer resulted in 100 per cent. control.

Hyalomma spp.

These ticks are considered difficult to kill with insecticides. The following results given in Table VIII indicate how adult males and females reacted in similar treatment to that described for *H. silacea*.

TABLE VIII.

Treatment	Stages of *Hyalomma* spp.	No. of ticks	% ♀♀ laid	% batches hatched	% control
Water ... ... 0·0062% gamma isomer ...	Fully engorged females	5 29	100 79	100 48	0 62
Water ... ... 0·0062% gamma isomer ...	Partly engorged females	5 21	80 24	50 40	60 90

On the day following treatment unengorged females and males treated in " Gammexane " were either dead or so affected that they were unable to crawl and all eventually died, none of the females laying. Those treated in water were alive, crawling actively and lived much longer than the ticks treated in " Gammexane ". One unengorged female treated in water even laid a small batch of eggs from which a few larvae hatched. The partly and fully engorged females treated in water were rounded and healthy, while those dipped in " Gammexane " showed the typical grooving and flattening which this insecticide causes.

It appeared from this experiment that female ticks could lay eggs in any stage of engorgement, but there was more chance of the female being fertilised and thus of the eggs hatching if they were laid by fully engorged ticks. As " Gammexane " was more effective against unengorged and partly engorged females, it would appear that cattle should be dipped more frequently than once per week to catch the newly attached adult female, which normally remains on the host for seven days.

I. pilosus.

The larvae and nymphs are found on small wild mammals and birds, the adults on domestic stock. Control measures must thus be directed against the adult as with most two- and three-host ticks.

The first indications that " Gammexane " was not wholly effective against this tick were noted during the initial field experiment in December, 1945. It was observed that while the other species of ticks were rapidly eliminated from the sprayed cattle, the paralysis tick seemed to persist and after four weekly sprayings adult *I. pilosus* were the only adult ticks to be found on the treated animals.

Dilutions of Formulation No. 2 were used in the spraying experiments, and when numbers of adult female *I. pilosus* became available they were treated in the laboratory, with the results given in Table IX.

The behaviour of *I. pilosus* in the laboratory was erratic and not consistent as in the case of *B. decoloratus*. Thus with the paralysis tick one sometimes found a high percentage lay and yet a small percentage hatch, resulting in a high degree of control even in the water treatment. Nevertheless these experiments indicated that :—

(1) Adult female *I. pilosus* tolerated a high concentration of " Gammexane ", while a low concentration killed *B. decoloratus*.

(2) Adult female *I. pilosus* were killed by 0·16 per cent. As_2O_3, while this concentration of arsenic had little effect upon *B. decoloratus*.

(3) Nicotine plus " Gammexane " killed *B. decoloratus*, but not *I. pilosus*, while arsenic plus " Gammexane " killed both species of tick.

Extensive field experiments were not carried out with species of ticks other than *B. decoloratus*, but the above observations indicated that "Gammexane" was toxic to five other species and might thus prove a useful adjunct to the normal arsenical dip.

TABLE IX.

Treatment	Exp. No.	Tick species	No. ticks	% ♀♀ laid	% batches hatched	% control
Water	55	*B. decoloratus*	30	87	69	40
,, ...		*I. pilosus*	30	50	40	80
0·16% As$_2$O$_3$		*B. decoloratus*	30	70	67	53
,, ,, ...		*I. pilosus*	30	0	0	100
Formulation No. 2 0·0045 % "Gammexane" ...		*B. decoloratus*	30	7	0	100
,, ,, ...		*I. pilosus*	30	47	21	90
Formulation No. 2 0·0045 % "Gammexane" +0·16 % As$_2$O$_3$		*B. decoloratus*	30	3	0	100
		I. pilosus	30	0	0	100
Water	66	*I. pilosus*	15	93	57	47
Formulation No. 2 0·0045 % "Gammexane" ...		,,	15	100	73	27
0·0051 % ,, ...		,,	15	80	33	73
0·0089 % ,, ...		,,	15	93	79	27
0·0178 % ,, ...		,,	15	93	29	73
0·16 % As$_2$O$_3$		,,	15	0	0	100
Water	80	*B. decoloratus*	10	100	90	10
,, ...		*I. pilosus*	10	90	100	10
Formulation No. 2 0·0045 % "Gammexane" ...		*B. decoloratus*	20	10	0	100
,, ,, ...		*I. pilosus*	20	85	94	20
Formulation No. 2 0·0045 % "Gammexane" +0·05 % Nicotine		*B. decoloratus*	80	18	7	99
		I. pilosus	80	65	75	49

Field Experiments.

Spraying cattle.

There was no available information regarding the toxicity of "Gammexane" dips to cattle, so five oxen were sprayed at weekly intervals with Formulation No. 2 diluted 1 in 120 to give a content of 0·0073 per cent. gamma isomer. The dip proved to be safe, for after three sprayings the oxen had improved in condition and, by their tick-free state, could readily be distinguished from the rest of the herd, which had remained undipped. The experiment was extended to include five milk cows, and after three weekly applications the animals were tick-free, except for *I. pilosus*. The most striking result was obtained when five grossly infested cows at the "Langholm Estates" were sprayed with Formulation No. 2 containing 0·0073 per cent. gamma isomer. Half an hour after spraying, one cow was made to stand on a canvas sail for an hour. In this period the following ectoparasites, representing only a portion of the total ectoparasite population, dropped off the cow: blue tick males, 1,473; fully engorged females, 130; partly engorged females, 197; nymphs, 49; larvae, 132: lice, 848.

All these parasites, particularly the normally active males, exhibited the characteristic twitching movements associated with the toxic action of "Gammexane". After twelve hours all the parasites, except the 130 fully engorged adult female blue ticks, were dead. These were placed in tubes and kept in the incubator. At the same time 100 fully engorged adult female blue ticks, which had been removed by hand from the cow, half an hour after spraying, were also kept in the incubator. The results of spraying ticks *in situ* are recorded in Table X, together with those of an *in vitro* application of the same diluted dip to adult female ticks.

TABLE X.

Treatment	No. ticks	Method of removal from host	% ♀♀ laid	% batches hatched	% control	
None	20	By hand	100	95	5 ⎫	*in situ* application
0·0073% gamma isomer ...	100	,,	86	83	29 ⎬	
,, ,, ...	130	Fell off	59	83	49 ⎭	
Water	80	By hand	98	97	5 ⎫	*in vitro* application
0·0073 % gamma isomer ...	80	,,	20	6	94 ⎭	

The results presented in Table X showed that the treatment of ticks *in situ* gave very different results from the *in vitro* application of dip, and also that the ticks which fell off were more affected than those removed by hand. If the latter had been allowed to remain on the host longer in contact with the "Gammexane" on the skin and hair, the results might have been very different. These results indicated the necessity for some standard method for treating ticks in the laboratory, so that the many complicating factors involved in the field treatment could be reduced to a minimum.

It also appeared from these results that a higher concentration of the gamma isomer might be necessary to control the ticks in the field. This was not the case, for in further spraying experiments with Formulation No. 2, it was found that 0·0045 per cent. and 0·003 per cent. gamma isomer contents were effective in keeping cattle free from all but the reinfesting larvae of the blue tick, if sprayed at weekly intervals. It was thus obvious that "Gammexane" was more toxic to larval ticks than to adult females, and that in the field the killing action would be more pronounced on the larvae. It also appeared that adult male ticks were more susceptible than adult females. This selective killing could result in the production of infertile eggs by the surviving females.

Dipping cattle.

Dipping trials were carried on for fifteen months, from March, 1946 to June, 1947. Nine dipping tanks were filled with "Gammexane" dips, six with emulsions and three with dispersible powders. In all cases the cattle were dipped at weekly intervals. In two tanks a composite wash, consisting of 0·16 per cent. As_2O_3 and "Gammexane" was tested. Weekly records were kept of the number of cattle that passed through each tank. The dip washes were replenished periodically, water and dip being added in a predetermined ratio. Specially prepared measuring sticks were used to check tank capacities, and in this way an accurate record was kept of all replenishments, including water additions by rain. At the end of each weekly dipping, samples of wash were taken for chemical analyses and for biological tests, using the *in vitro* technique for adult female ticks. At each dipping tank a small group of not more than ten undipped animals was kept as a control, and just before each weekly

D

dipping, scrapings were taken from the neck of one. At the same time scrapings were taken from the neck of a dipped animal. The neck was chosen as the site for scraping as this area was most favoured by the blue tick. If scrapings had been taken from elsewhere, other tick species might have occurred in greater numbers among the ectoparasites removed. The ectoparasites collected in this way were later classified. A metal grape fruit knife was used to make these scrapings, as the blunt serrations proved ideal for removing all stages of ticks and lice.

Laboratory examinations of dip wash samples.

Much information was gathered from the nine experimental dipping tanks. The results of chemical analyses of dip wash samples from one tank and *in vitro* biological tests with adult female blue ticks with the same samples, are given in Table XI.

From Table XI it will be seen that as time passed and as the number of cattle dipped increased, so the estimated gamma isomer content of the wash dropped. The *in vitro* percentage control of adult ticks also decreased. On each occasion when the tank was replenished, this was reflected in the chemical analyses as well as in the biological tests.

These results are representative of those obtained at the other experimental dipping tanks. The drop in the estimated gamma isomer content* is attributed to the removal, by the skin and hair of the animals, of the oil phase of the dip wash, as there was no evidence of the chemical decomposition of the hexachlorocyclohexane. There were, however, indications that the biological efficiency of the washes decreased as they became older, but the reasons for this are not fully understood. In spite of this loss of " Gammexane ", which was reflected in the poor results of the *in vitro* biological tests on adult female ticks, the dip washes were effective in controlling the arsenic-resistant blue tick in the field, thus confirming the results of spraying experiments previously obtained with Formulation No. 2.

Field evaluation of " Gammexane " dips.

The scrapings of ectoparasites taken from the necks of undipped and dipped animals furnish striking evidence of the effectiveness of the " Gammexane " dips. The ectoparasites were sorted into groups consisting of (1) males (2) females (3) nymphs and (4) larvae of the one host blue tick, (5) other tick species and (6) lice. These data, relating to some of the experimental tanks, are presented in a series of histographs (figs. 3 to 6). Actual counts of ectoparasites are represented on the vertical axes, while the horizontal axes are divided into months showing the number of weekly scrapings taken during the course of the experiment. A gap on this axis indicates that a weekly dipping was missed.

" *Langholm Estates* ". *Tank 1.*—An emulsion dip, similar to Formulation No. 27, was used in this tank. The experiment ran from 7th May, 1946 to 20th May, 1947, and during this period fifty-two samples of wash and scrapings were taken. An average of 494 cattle were dipped each week and the dip was used at a dilution of 1 in 400 from 7th May, 1946 to 11th March, 1947, with an original gamma isomer content of 0·005 per cent., but for most of the dippings the wash was much below this strength. On 18th March, 1947, the tank was fortified to bring the content to 0·005 per cent. and was subsequently replenished at 1 in 250, but even this rate of replenishment failed to maintain a content of 0·005 per cent.

The analyses of scrapings from the undipped animals and those dipped in this tank are given in fig. 3.

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*From here onwards all references to the " Gammexane " or gamma isomer content are estimated and not actual.

TABLE XI.

Chemical and biological data relating to dip samples taken from "Faberskraal" (G. C. Mullins).

Sample Date 1946	Replenishments		No. cattle dipped	Chemical analyses*		% ♀♀ laid	Biological Tests		
	Water gals.	Dip pts.		% BHC	Estimated % "Gammexane"		% batches hatched	% control	Water % control
20 May	3,700	29⅗	435	0·014	0·0047	15	0	100	20
28 ,,	—	—	406	0·011	0·0037	10	0	100	0
4 June	—	—	435	0·007	0·0024	75	67	50	0
12 ,,	875	7	430	0·007	0·0024	60	50	70	10
18 ,,	—	—	410	0·007	0·0024	40	88	65	0
25 ,,	—	—	436	0·005	0·0017	75	73	45	10
2 July	—	—	416	0·006	0·0020	90	89	20	0
9 ,,	—	—	440	0·005	0·0017	80	81	35	0
16 ,,	—	—	400	0·004	0·0013	90	94	15	0
23 ,,	1,175	9²	430	0·007	0·0024	45	78	65	0
30 ,,	—	—	410	0·006	0·0020	95	89	15	0
6 Aug.	—	—	510	0·004	0·0013	85	82	30	0
13 ,,	—	—	402	0·006	0·0020	95	84	20	0
20 ,,	—	—	410	0·007	0·0024	95	100	5	0
27 ,,	1,500	12	410	0·009	0·0030	55	55	70	0
3 Sept.	—	—	380	0·007	0·0024	75	67	50	0
10 ,,	—	—	370	0·006	0·0020	90	67	40	0
17 ,,	—	—	402	0·004	0·0013	100	95	5	0
24 ,,	—	—	370	—	—	100	95	5	0
1 Oct.	750	6	402	—	—	75	60	55	10
8 ,,	—	—	350	0·004	0·0013	100	90	10	0
17 ,,	—	—	402	—	—	95	84	20	0
22 ,,	—	—	370	—	—	100	100	0	0
31 ,,	—	—	347	0·002	0·0007	100	100	0	0
5 Nov.	700	5⅗	345	0·005	0·0017	70	86	40	0
12 ,,	—	—	316	0·002	0·0007	95	100	5	0
19 ,,	—	—	331	0·002	0·0007	100	100	0	0
28 ,,	—	—	273	0·002	0·0007	100	100	0	0
3 Dec.	800	8⅖	330	0·007	0·0024	90	89	20	10

Dip No. 28 diluted 1 gal. to 1,000 gals. water.   Twenty ticks treated in each sample and in water.

*The results of chemical analyses on samples taken during the months of June to September, inclusive, are unreliable, as it was subsequently found that the fouling of the tank interfered with the analytical method then in use.

D²

This shows that after three weekly dippings the cattle were free from all ectoparasites except larvae of the blue tick, and remained in this condition for a while. Later some of the larvae which attached themselves to the dipped animals developed further, and a few males, females and nymphs appeared. At the end of the experiment the dipped animals had been free from these for seven weeks, showing
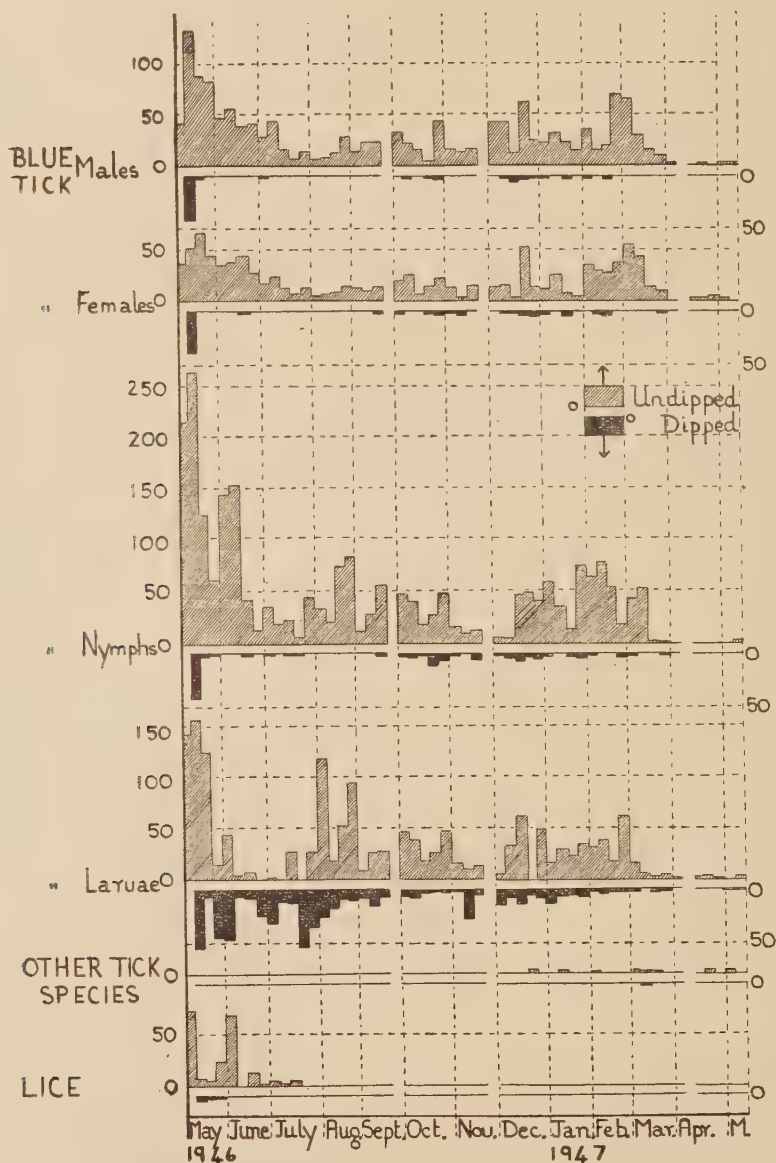
Fig. 3.—Diagrammatic representation of the control effect of " Gammexane " emulsion dip on the ectoparasites of cattle dipped at weekly intervals. Four hundred and ninety-four cattle dipped each week. Parasites from undipped animals above lines, from dipped animals below lines.

that in spite of the low concentration of "Gammexane" an effective control of the arsenic-resistant blue tick had been obtained in the field. The undipped animals remained heavily infested with all stages of the blue tick until the end of March, 1947. After this, the undipped animals were relatively free from ticks, indicating that the blue tick population on this portion of the "Langholm Estates" had been markedly reduced.

Fig. 3 also illustrates the seasonal occurrence of lice on cattle, and shows that after the third weekly dipping, "Gammexane" completely eliminated these parasites.

"*Langholm Estates*". *Tank 2*.—The wash in this tank consisted of the same "Gammexane" emulsion dip as used in Tank 1, diluted 1 in 400. To this was added 2 lb. arsenite of soda per 100 gallons, which gave 0·16 per cent. As_2O_3, the recognised 7-day strength of an arsenical wash. The number of cattle dipped each week varied from 354 to 688 with an average of 455, and the experiment ran from 7th May, 1946 to 3rd April, 1947. The original content of this composite wash was 0·005 per cent. gamma isomer but as in the case of all the other experimental dips this dropped as the number of cattle treated increased. In spite of this the rate of replenishment was maintained throughout the experiment at 1 gallon dip to 400 gallons water, plus 2 lb. arsenite of soda per 100 gallons water. As with Tank 1 this rate of replenishment did not maintain a gamma isomer content at 0·005 per cent., and at times it dropped as low as 0·001 per cent.

The results obtained from examinations of the scrapings from the dipped and undipped animals at this tank are represented in fig. 4.

After three weekly dippings all stages of the blue tick except larvae, were eliminated from the dipped animals, but later a few nymphs, females and males appeared. By the beginning of October, 1946, after five months of weekly dippings, there was a marked drop in the blue tick population on this section of the "Langholm Estates", and from then on the arsenic-resistant blue tick ceased to be a menace.

The heavy seasonal infestation of lice on cattle at this tank is shown in the scrapings taken from the undipped animal. Four weekly dippings completely eliminated the lice.

The addition of arsenic to the "Gammexane" wash in Tank 2, did not show any advantage over the wash in Tank 1, containing no arsenic. Other species of ticks, against which a dip containing 0·16 per cent. As_2O_3 is effective, were not plentiful on cattle at either of the "Langholm" tanks, but the arsenic-resistant blue tick was effectively controlled by a concentration of "Gammexane" much below 0·005 per cent. for most dippings.

"*Lyndhurst*." *D.A.L. Dold*. The wash in this tank contained 0·16 per cent. As_2O_3 in combination with "Gammexane". From 28th May, 1946 to 10th September, 1946, emulsion Formulation No. 2 was used diluted 1 in 200 with an original gamma isomer content of 0·0039 per cent. From 17th September, 1946, until the experiment was discontinued on 8th October, 1946, replenishments were made with Formulation No. 27, diluted 1 in 600, but at no time did the gamma isomer content equal 0·005 per cent.

One hundred and twenty-five cattle were dipped in this tank each week, and the striking effect of this treatment upon the arsenic-resistant blue tick is illustrated in fig. 5.

After three weekly dippings only larval blue ticks appeared on the dipped animals, while the controls remained grossly infested with all stages of the tick for five months, until the experiment was discontinued.
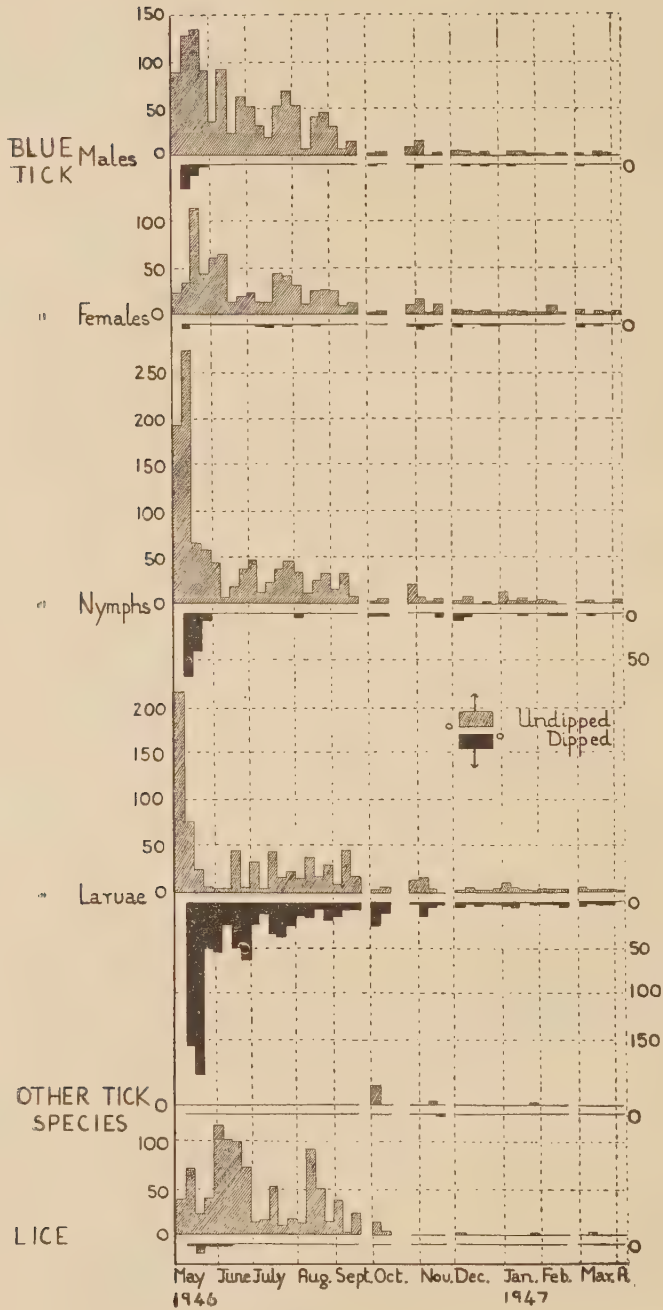
Fig. 4.—Diagrammatic representation of the control effect of "Gammexane" emulsion dip plus 0·16 per cent. As$_2$O$_3$ on the ectoparasites of cattle dipped at weekly intervals. Four hundred and fifty-five cattle dipped each week.

The *in vitro* biological tests with samples of wash from this tank gave consistently high percentage controls against adult female blue ticks, and thus confirmed the results depicted in fig. 5. Chemical analyses showed a drop from 0·0039 per cent. to 0·0015 per cent. in the gamma isomer content, but this was not so rapid as in tanks dealing with larger numbers of cattle per week. The results from this tank indicated that the fewer the cattle dipped, the longer the wash would last, and confirmed the opinion that the drop in gamma isomer content was not due to any chemical decomposition, but rather to a preferent removal of the insecticide on the skin and hair of the animals.
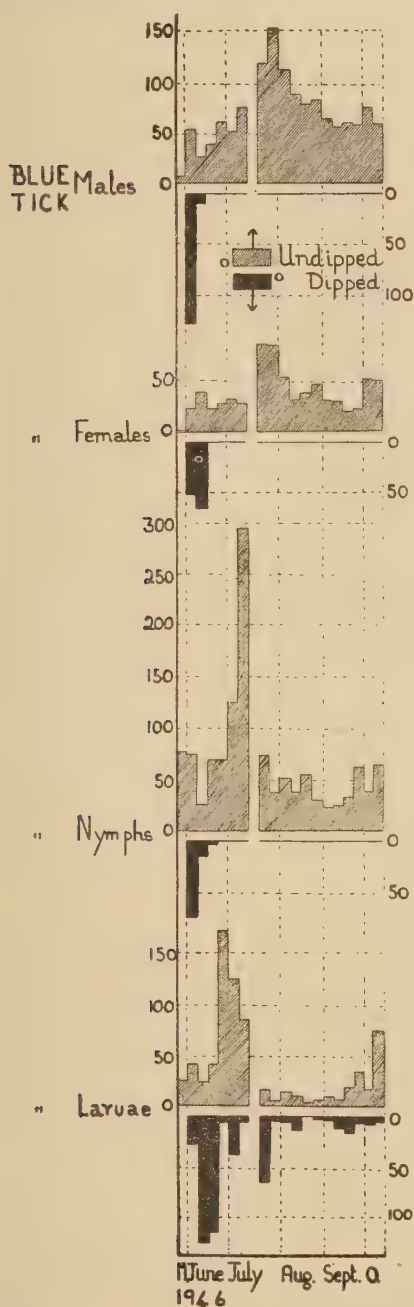
" *Paardekraal* ". Ford Bros. A dispersible powder dip, consisting of a benzene extract of crude hexachlorocyclohexane deposited on china clay, was used in this tank throughout the experiment at the rate of 1 lb. per 100 gals. The average number of cattle dipped each week was 416, and the experiment continued from 23rd September, 1946 to 13th March, 1947. Samples of dip wash taken during the first three weeks of the experiment from the top, middle and bottom of the tank before dipping, all gave about 40 per cent. control of adult female blue ticks in *in vitro* tests, compared with 100 per cent. control in a sample taken after ten cattle had stirred the wash. Half an hour after dipping, samples from the top, middle and bottom gave 40 per cent., 60 per cent. and 70 per cent. control respectively, indicating a rapid settling out of the dispersible powder. The original content of the wash was 0·0039 per cent. gamma isomer, but like the emulsion dips, chemical analyses and *in vitro* biological tests with wash samples, indicated that a dispersible powder wash could also decrease in gamma isomer content as the number of cattle dipped increased. Nevertheless, normal replenishments of 1 lb. dip per 100 gals. water, maintained the toxicity sufficiently to control infestations of the blue tick in the field. The analyses of scrapings from cattle at this tank are shown in fig. 6.

The blue tick infestation on " Paardekraal " at first appeared light, but later many larvae attached themselves to both dipped and undipped animals. For a period, the dipped cattle remained quite free from males, females and nymphs, while the undipped animals became grossly infested with all stages of the blue tick. When the experiment was discontinued in March, 1947, the blue tick population on " Paardekraal " had been greatly reduced.

Fig. 5.—Diagrammatic representation of the control effect of " Gammexane " emulsion dip on the arsenic-resistant blue tick on cattle dipped at weekly intervals. One hundred and twenty-five cattle dipped each week. Dip wash in tank dealing with few cattle remains effective longer.

Figs. 3 to 6 illustrate that all cattle, whether dipped or undipped, picked up blue tick larvae from the pastures. Larvae from the dipped animals however were only five-sevenths of the size of those from the undipped animals. Furthermore, only a few larvae picked up by the dipped animals developed into nymphs and adults, demonstrating that weekly dippings in "Gammexane" controlled this single host tick mainly through the larval stage. In most cases six to eight months of weekly dippings brought the arsenic-resistant blue tick under control, for after that time the undipped animals were almost as tick-free as the dipped animals, indicating an absence of blue tick life on the pastures.

As depicted in figs. 3 to 6, other tick species appear to be of minor importance. This is due to the technique used which was designed principally to follow the effect of dipping on the blue tick. Thus, as stated previously, if areas other than the neck regions of the animals had been scraped, other tick species might have occurred in greater numbers among the ectoparasites removed. In all cases, however, the arsenic-resistant tick, a transmitter of piroplasmosis and anaplasmosis, was the predominant parasite encountered, and its economic importance is shown in Table XII, which records the monthly rainfall and deaths of cattle for the years 1945 to 1947 on a ranch in the Eastern Cape.

Fig. 6.—Diagrammatic representation of the control effect of "Gammexane" in suspension form (powder dip) on the ectoparasites of cattle dipped at weekly intervals. Four hundred and sixteen cattle dipped each week.

TABLE XII.

Rainfall and cattle mortality figures on Large Ranch. "Gammexane" dips first used on 7th May, 1946.

Month	1945			1946			1947		
	Rainfall inches	No. cattle died	% deaths	Rainfall inches	No. cattle died	% deaths	Rainfall inches	No. cattle died	% deaths
Jan. ...	2·37	17	0·86	0·83	45	3·63	1·54	7	0·57
Feb. ...	1·40	18	0·95	1·37	24	2·00	1·63	7	0·57
March ...	0·61	13	0·69	3·43	54	4·51	3·63	17	1·35
April ...	0·55	39	2·12	1·22	38	3·39	1·88	1	0·08
May ...	0·72	129	6·86	0·59	7	0·62	1·18	2	0·15
June ...	1·32	201	13·53	0·31	4	0·36	2·05	2	0·14
July ...	0·40	38	2·92	0·97	6	0·52	1·35	2	0·14
Aug. ...	0·55	12	0·96	0·60	8	0·68	0·10	2	0·14
Sept. ...	0·75	22	1·80	1·03	4	0·29	1·25	2	0·14
Oct. ...	2·46	17	1·34	2·04	2	0·16	1·29	8	0·52
Nov. ...	0·30	8	0·62	1·30	0	0·00	2·63	9	0·52
Dec. ...	0·47	34	2·67	1·83	6	0·50	0·86	4	0·27
Total ...	11·90	548	35·32	15·52	198	16·66	19·39	63	4·59

1945 and 1946 were years of severe drought, with total rainfalls of only 11·90 and 15·52 ins. respectively, compared with an average annual rainfall of 24 ins. Table XII shows how cattle continued to die in large numbers until April, 1946. Previously they were dipped in arsenic, or in the case of some herds, not dipped at all. From May, 1946, the cattle were dipped at weekly intervals in "Gammexane" wash, but otherwise received the same treatment as before. The number of deaths was immediately reduced, although the drought continued. Reports of similar results from the use of "Gammexane" were received from all over the Eastern Cape and Natal, where the effective control of the arsenic-resistant blue tick safe-guarded the cattle industry.

Summary.

The rapid spread of the single host arsenic-resistant blue tick, *B. decoloratus*, a transmitter of piroplasmosis and anaplasmosis, seriously affected a large and important cattle ranching area in South Africa.

Biological tests, using the laboratory technique described in an earlier paper, enabled the most effective "Gammexane" preparations to be rapidly selected from numbers of experimental dips. The technique was also employed as a supplement to chemical analyses of dip wash samples where only the total hexachlorocyclohexane and not gamma isomer was estimated. Twenty-thousand adult female ticks were used in these *in vitro* tests. Oil emulsions seemed more active than dispersible powders or pastes, but all tests indicated that 50 parts per million "Gammexane" should satisfactorily control the tick in the field.

Laboratory tests with larvae of the blue tick showed that "Gammexane" had a persistent action and was very toxic to this stage of the tick. Excellent control of larval ticks could be anticipated in the field.

In vitro tests indicated that "Gammexane" was effective against the two- and three-host ticks, *R. evertsi*, *R. appendiculatus*, *A. hebraeum*, *H. silacea* and *Hyalomma* spp., but hand dressing and shorter dipping intervals might be necessary to control these ticks in the field. The sheep paralysis tick, *I. pilosus*, seemed to be resistant to "Gammexane" but was readily killed by arsenic.

Preliminary spraying tests indicated that "Gammexane" would not injure cattle, but had a rapid action on ticks and lice, and markedly reduced infestations of these ectoparasites.

Nine dipping tanks were filled with experimental "Gammexane" dips, and were kept under observation for fifteen months. Chemical analyses of dip wash samples indicated that there was a drop in the gamma isomer content as the number of cattle dipped increased. Biological tests with the same dip wash samples confirmed this. They also showed how normal replenishments increased the toxicity of the wash and maintained it at sufficient strength to control the single host blue tick in the field. Scrapings of ectoparasites from dipped and undipped cattle showed that all cattle picked up larvae from the pastures, but these developed into nymphs and adults only on the undipped animals. It has been shown that weekly dippings in "Gammexane" eventually brought the arsenic-resistant blue tick under control. This knowledge made it possible to safeguard the cattle industry in the Eastern Cape and Natal.

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We wish to acknowledge the hospitality granted by the Zoology and Chemistry Departments of Rhodes University College, Grahamstown, S. Africa. The biological work was carried out in the former, while Mr. E. F. Meerholz completed the series of chemical analyses in the latter. The experimental dips were prepared by Dr. I. McGillivray, Dr. W. C. Walmsley and Mr. F. de Wilde, to whom we are indebted for much helpful advice.

The following farmers from the Albany and Bathurst Districts placed dipping tanks and cattle at our disposal and assisted with the field work : Mr. D. A. L. Dold, " Lyndhurst " ; Messrs. H. J. and D. Ford, " Paardekraal " ; Mr. G. V. Ford, " Glendower " ; Mr. F. P. Ford, " Elmhirst " ; Mr. A. W. Legg, " Langholm Estates " ; Mr. G. C. Mullins, " Faberskraal " ; Mr. R. N. Mullins, " Assegaai Bush East " ; and Mr. V. Webb. " Tharfield ".

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STUDIES ON THE ATTRACTIVENESS OF HUMAN POPULATIONS TO ANOPHELINES.

By C. R. RIBBANDS.

Rothamsted Experimental Station, Harpenden, Herts.

These experiments were made in West Africa during 1941 and 1942, while the author was a member of No. 5 Malaria Field Laboratory, R.A.M.C.; they represent attempts to estimate some of the effects of quantitative changes in human populations upon the distribution of their attendant Anophelines. Some of these experiments were conducted at Aberdeen, near Freetown, Sierra Leone, in huts which attracted a population of *Anopheles melas*, Theo., and others were made at Krabonekrom, near Sekondi, Gold Coast, in an area infested with *A. funestus*, Giles. Both localities were the subject of other experiments, and the method of catching the mosquitos has already been recorded (Ribbands, 1946a, b). A preliminary report of some of the present results has already been given (Ribbands, 1946d).

Fluctuations in Human Attractiveness.

An experiment was conducted on the *A. melas* population at Aberdeen, in which three Africans slept in separate huts, under supervision, and the mosquitos attracted to them each night were killed by pyrethrum insecticide, collected, and recorded. Each individual slept in turn in a different hut each night. Analysis of the results failed to show any significant differences between the attractiveness of the huts, and so it has been possible to eliminate this factor.

The marked repellent effects of the insecticide (Ribbands, 1946c; Thomson, 1947) can also be ignored, as they too were evenly distributed and could not have produced the present results. The three Africans were present on 49 days between 2nd August and 26th September 1941. The seven days during this period when no results are given were occasions when one or more of the three Africans was absent. A fourth African was present between 4th and 26th September.

Every female *A. melas* caught from each man during this period is recorded in Table I, and the sequences of the largest catches are shown in fig. 1.

Fig. 1.—Sequence of largest catches of *A. melas*. Black squares represent largest catches, dots represent largest catch from either A, B or C.

The total catches of A, B and C during the whole period were not very dissimilar (A, 1,068, B, 902, and C, 918). Despite this there were periods of several successive days, during which one or other attracted considerably more than either of the others. For eight days between 11th and 19th August C was consistently most attractive and collected 63 per cent. more mosquitos than B, his nearest rival. For four days between 23rd and 27th August A was consistently most attractive, and collected 58 per cent. more than B, who was again the runner-up. For another nine days between 29th August and 6th September, with one exception, B was most attractive, and in this period he attracted 146 per cent. more than A and 198 per

C. R. RIBBANDS.

TABLE I.

Daily catches of *Anopheles melas*, from each individual.

August

Date	2	3	4	5	6	7	8	9	11	12	13	14	15	17	18	19	20	21	22	23	25	26	27	29	30	31
A.	15	20	8	2	8	7	20	20	24	13	20	22	19	15	15	9	10	18	16	18	27	18	27	4	5	4
B.	14	9	8	4	4	7	21	26	27	28	14	23	13	11	17	16	5	20	11	16	22	10	8	4	25	45
C.	13	19	3	7	5	11	26	20	55	45	26	33	22	15	17	24	4	17	20	10	22	10	2	3	0	9
Non-random distributions									*	*													*		*	*

September

Date	1	2	3	4	5	6	7	8	10	11	13	15	17	18	19	20	21	22	23	24	25	26
A.	6	5	11	5	26	12	1	12	23	43	21	41	17	51	95	28	55	16	33	21	5	28
B.	13	12	7	20	44	16	6	28	4	42	23	17	34	83	26	25	27	12	9	10	10	12
C.	9	9	7	9	6	12	14	55	5	81	41	10	25	42	28	32	27	3	14	17	10	12
D.				31	75	59	35	118	33	31	86	129	86	37	61	21	35	17			29	25
Non-random distributions				*	*	*	*	*	*	*	*	*	*	*	*		*		*	*	*	*

cent. more than C. After a further period of short fluctuations with all three dominant in turn, A again became most attractive, and from 19th to 26th September he produced the biggest catch on six days out of eight, and collected 98 per cent. more mosquitos than C, the runner-up. An additional man, D, was introduced into the experiment on 4th September, and he attracted a bigger catch than either A, B, or C on nine out of the following ten days; in this period he attracted 196 per cent. more than the average catch of A, B, and C together ; he then became relatively less attractive, and attracted the largest catch on only two occasions out of the subsequent nine.

I am grateful to Dr. J. Martin, of the Statistical Department, The London School of Hygiene, for examining the distribution of these catches. He has pointed out that, using the criterion that the expected catch on any day is the mean of all the catches on that day, the distribution on five of the first 29 days differed significantly from a chance one, and that for the last 19 days, when four huts were in use, the distribution of catches differed from a chance one on every day except one (Table I). In addition, the sequence of largest catches (fig. 1) was certainly not a chance one. On the first 29 days there were three days when two individuals both obtained the maximum catch; if each man had attracted mosquitos equally the largest daily catch would have been distributed at random, and the chance that any individual had the largest catch on any one day would have been 32/87. The chance of getting the largest catch for n consecutive days would have been $(32/87)$ n, so that the chance of getting the largest catch five days running (A, 23rd to 29th August ; B, 29th August to 2nd September) would have been 0·007, and of getting the largest catch eight days running (C, 11th to 19th August) 0·0003. A similar calculation shows that in the last 19 days the chance of getting the largest catch six days running (D, 4th to 10th September) would have been 0·0003.

These data indicate that the large catches tended to occur in groups much more often than would be expected on the hypothesis that a large catch on one day was unrelated to the size of the catch on adjacent days.

De Meillon (1935) showed that attractiveness to *A. funestus* was considerably diminished after thorough deodorisation with soap and water, and Haddow (1942) demonstrated that a group of unwashed children attracted more mosquitos than a similar group of washed children, but this factor is hardly sufficient to explain the variation revealed in the present results. The Africans bathed several times each week ; in addition, if washing had been the only factor involved, the distribution of the catches might have been expected to be rather different, with sudden decreases and gradual increases in individual attractiveness.

The results prove that mosquitos can discriminate between the attractiveness of different individuals, and demonstrate that there are marked fluctuations in the attractiveness of the same individual to mosquitos ; they provide no evidence of the cause of these fluctuations, but the work of De Meillon and Haddow indicates that they are likely to be due to a variation in the quality or quantity of perspiration.

The experiment demonstrates a method by which human attractiveness to mosquitos can be investigated, and it would be interesting to conduct a more extensive series of experiments in an attempt to correlate the fluctuations in attractiveness with the physiological conditions of the human host.

Despite the marked fluctuations in individual attractiveness, which demonstrate the power of discrimination possessed by the mosquitos, the results showed no consistent preference on their part for either A, B or C. But examination of the available data subsequent to this experiment showed that D, who was very attractive when introduced into the experiment on 4th September, did maintain his superior attraction fairly consistently, and 16-day averages showed him to be more attractive than A, B or C until the end of November, when catches ceased. The data for this period were recorded as 3 : 1 ratios (see below) and are therefore not reproducible on a daily basis.

It is likely that if men of different races, feeding habits, and behaviour were the subject of similar experiments considerable variations in their attractiveness to mosquitos would be demonstrated.

Montgomery (1932) stated that daily ingestion of sulphur in quantities sufficient " to colourise sweat " prevented mosquitos from biting, a claim partially confirmed by De Meillon (1935), who found that eight days on a sulphur diet reduced the number of *A. funestus* which fed. It is therefore possible that there is justification for a prevalent belief, mentioned by Bristowe (1946), that some individuals are so unattractive to mosquitos that they can expose themselves with impunity, but probably such claims are usually made by fortunate individuals who have escaped the more obvious consequences of mosquito bites (Mellanby, 1946) and have therefore erroneously concluded that they have never been bitten.

Relative Attractiveness of one Man and several Men.

(*a*) To *A. melas* females.

On 57 days between 2nd October and 27th November, 1941, the four individuals already mentioned slept in two of the huts at Aberdeen, and it was arranged that three men would sleep in one hut, and the fourth in another. Each man took his turn to sleep alone, and in this way the effects of different attractiveness of the individuals were largely counterbalanced.

The results can be summarised thus :—

A alone, 45 *A. melas* ; other three on same night, 145 ; A's proportion, 22·6 per cent.

B alone, 78 *A. melas* ; other three on same night, 222 ; B's proportion, 26 per cent.

C alone, 46 *A. melas* ; other three on same night, 139 ; C's proportion, 24·8 per cent.

D alone, 54 *A. melas* ; other three on same night, 103 ; D's proportion, 34·2 per cent.

The average proportion obtained by one man was 27 per cent. of the total catch by all four ; the ratio between the number of *A. melas* caught by one man and the number caught by three men was 1 : 2·72.

(*b*) To *A. funestus* females.

This experiment was conducted at Krabonekrom, a village near Sekondi, Gold Coast. Seven Africans were employed and the experiment was made in Hut Y, consisting of a row of three rooms, each approximately 20 ft. × 15 ft. There were communicating doors between the three rooms and an external door to the central room which was never used. Three men slept in one end room, and one man in the other. The men were selected in rotation from the seven men, so that variations in human attractiveness were cancelled out, and any effect of the rooms themselves was cancelled by reversal on alternate nights.

The experiment continued for 40 days from 3rd February to 16th March, 1942, and the results are shown in the histogram in fig. 2.

During five of these days Anopheline discrimination was affected by clearance of the bush which surrounded the huts and the hut containing one man then attracted a considerably higher proportion of the total mosquitos than at other times (Ribbands, 1946b). During the remaining 35 days 1,109 female *A. funestus* were collected from the two rooms, of which 338 had been attracted to the one man and 771 to the three men. Thus the average proportion obtained by one man was 31 per cent. of the total catch of all four, and the ratio between the number caught by one

man and by three men was 1 : 2·28.   On only one of these 35 days did the catch of the one man exceed that of the three men, and on only one other occasion was it equal to this catch—these two occasions were towards the end of the experiment, when the mosquito population was smaller, and the margin of error therefore likely to be greater.
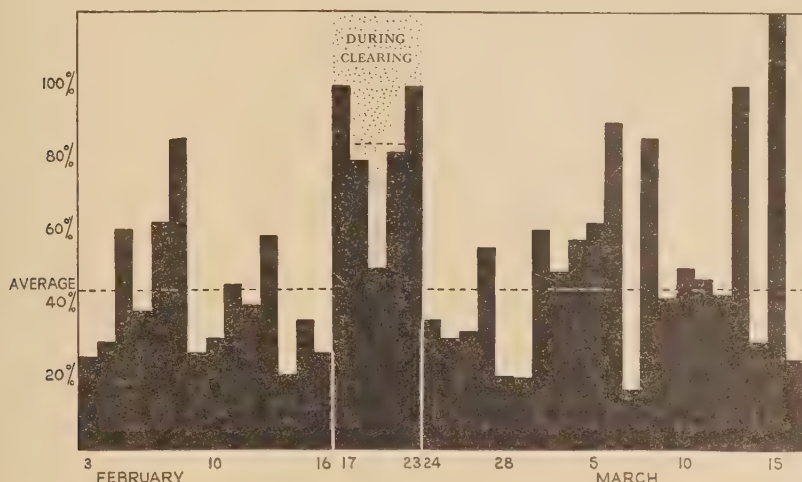
Fig. 2.—Daily catches of *A. funestus*.   Catch from one man, expressed as proportion of catch from three men.

(c)   To *A. gambiae* females.

There was also a small population of *Anopheles gambiae*, Giles, at Krabonekrom, and over the same 35 days 122 females of this species were collected, of which 36 came from the hut which contained one man.   One man therefore obtained 29·5 per cent. of the total catch of all four, and the ratio between the number caught by one man and by three men was 1 : 2·38.

These results can be compared with those which have been reported from East Africa by Haddow (1942) who compared catches of *A. funestus*, *A. gambiae* and several species of Culicines obtained from huts containing one, two, five, ten and 15 African boys.

Haddow's results indicated that *A. funestus* and *A. gambiae* females did not, apparently, discriminate between one and two individuals ; but that more were attracted to larger numbers of boys.   From his Table XXV one can calculate that the ratio of mosquitos attracted by one boy to those attracted by five boys were, for *A. funestus* females 1 : 1·46, and, for *A. gambiae* females, 1 : 1·36.   For larger numbers the ratios rise more steeply, and the ratios for those attracted to five boys to those attracted to 15 boys were, for *A. funestus* females 1 : 2·56, and for *A. gambiae* females 1 : 2·8.

Haddow's ratios for catches of five and 15 boys are very similar to the present ratios for catches of one and three men, yet his results when small numbers (one, two and five) of boys were used do not demonstrate the same quantitative relationship. The difference between these results is attributed to the fact that Haddow's mosquitos were hand caught from unsprayed huts, while in the experiments recorded here they were all obtained by daily pyrethrum spraying.   This rendered the huts highly repellent to mosquitos (Ribbands, 1946c ;   Thomson, 1947) and it is believed that during the present experiments in West Africa this repellent effect was sufficient

to prevent nearly all random wandering into huts for shelter, although frequently not sufficient to prevent entry by hungry females which had scented food. (This would explain why Haddow recorded a much higher proportion of unfed females and much greater numbers of males in his catches.) The discrepancy between the results could be explained if it were supposed that many of the Anophelines found by Haddow in huts containing one boy only were random wanderers, which had perhaps entered for shelter before commencing their quest for food, whereas pyrethrum repellency prevented random wandering. With increasing numbers of boys used as bait the proportion of random wanderers would diminish, and therefore Haddow's more crowded huts indicate an Anopheline discrimination of similar order to that shown here. The hypothesis receives strong support from an analysis of Haddow's catches of *Taeniorhynchus africanus*, Theo., and *T. uniformis*, Theo. These two species are both non-shelterers, and are only found in houses when they enter to feed. Most of them leave soon after feeding. Haddow's results show that both species could readily discriminate between one and two natives and that catches were approximately proportional to the total number of boys ; the catches from one, five, ten and 15 boys were 12, 84, 136 and 169 respectively, equivalent to ratios of 1 : 7 : 11·3 : 14·1. It has already been pointed out (Ribbands 1946a) that comparison of night and day catches reveals that in empty huts there were much higher proportions of female Anophelines in night catches, and it was stated " that this fact affords evidence that huts are not preferred resting places for unfed female Anophelines, which enter to search for food and usually depart if they fail to find it ". The present evidence suggests that this view should be modified and that a proportion of them are probably first attracted to the huts as resting places, but become hungry and seek food later in the night, and depart if they fail to find it.

Relation between Anopheline Infestation and Population Distribution.

(1) *Effect of village removal.*

This experiment was conducted at Krabonekrom, a mosquito-ridden village of 26 huts, that had to be pulled down because it was a source of infection for a nearby military hospital. The village was situated on one side of a gently sloping valley, and the hospital was on a small hill about 500 yards away (see fig. 3). A preliminary survey of the district, conducted in mid-December, 1941, indicated the following room indices in selected rooms ; Krabonekrom, 322 ♀ *A. funestus*, 23 ♀ *A. gambiae* ; Asamang, 117 ♀ *A. funestus*, 8 ♀ *A. gambiae* ; Nkontompo, 249 ♀ *A. funestus*, 22 ♀ *A. gambiae* ; Dokolo, 11 ♀ *A. funestus*, 5 ♀ *A. gambiae* ; Fijai, 49 ♀ *A. funestus*, 5 ♀ *A. gambiae*. The rainy season had then finished, and the streams were drying up. No larvae were found in the canalised stream which commenced near Fijai and flowed past Krabonekrom, and the chain of swamps in the vicinity of Nkontompo were brackish, and harboured no *A. funestus*. The two large swamps on either side of Asamang were the only breeding grounds found in the vicinity. *A. funestus* larvae were very abundant in the upper portions of both swamps, and *A. gambiae* larvae were fairly plentiful in their middle and lower portions. The easy nature of the terrain rendered it most unlikely that any important breeding ground would be overlooked, and it was therefore concluded that the very large mosquito population at Krabonekrom was derived from the swamp which lay between there and Asamang. The higher room index at Krabonekrom than at Asamang was considered to be a consequence of the smaller size of the Krabonekrom community. When the experiment was commenced the breeding grounds were drying up, and the mosquito population everywhere was considerably less than it had been during the mid-December survey.

The experiment was designed to compare mosquito catches in the village with those in the surrounding district, both before and after the removal of the village.

Fig. 3.—Plan of Krabonekrom and district.

Two huts on the swamp side of the village were requisitioned before the removal began, and seven African labourers were paid to sleep regularly in them. A third uncompleted hut, adjoining these two, was used for supervision purposes ; Sgt. Eames, R.A.M.C., and the writer shared this duty and one or the other slept there each night. One of the Africans slept alone, the others slept in two groups of three, and experiments already described were being conducted simultaneously. For the present experiment it is legitimate to group together, for each night, the extra catch from all seven of the Africans, and this catch will be referred to as the Experimental Catch.

Another hut was specially built for the experiment 190 yards away from the village, on the direct line between it and the swamp. The same three Africans slept in this hut every night, and attracted the Inner Control Catch.

The Outer Control Catch was provided from three catching stations within the hospital precincts. One of these huts was occupied by three African orderlies, and the other two by one African servant each. (One of these latter subsequently had to be abandoned. With this exception the catching stations were those used in the bush clearance experiment performed afterwards and already described, Ribbands, 1946b.)

Daily catches were made in all of these huts for one week, and on the seventh day every hut in the village was pulled down except the two which provided the Experimental Catch and the one used for supervision. Daily catching was continued uninterruptedly after this time.

The very great natural short-term fluctuations already recorded in this mosquito population (Ribbands, 1944a) would invalidate any judgment of the effect of village removal in terms of the absolute numbers of mosquitos entering the area before and after removal, and effects can only be judged in terms of the proportion of the local mosquito population which entered. With this handicap the results probably considerably underestimate the changes which took place, because many of the mosquitos which would have been attracted to the village probably went instead to the hospital, which was the main alternative food source, and thereby increased the catches in the Outer Control Huts and lowered the proportions found in the other huts.

Nevertheless, the catches in the Outer Control Huts were the best obtainable measure of the local mosquito population, and therefore in all cases the other catches have been expressed as percentages of the catches in these huts at the same time.

The haste with which the experiment had to be designed made it impossible for an adequate number of catches to be made before the village was removed. In particular, the Inner Control Catch was obtained from a newly-built hut, and the accumulation of residual human scents within it may have caused its attraction to increase during the first week or so of the experiment. Because of these handicaps only a few conclusions can justifiably be drawn.

Table II records the total weekly catches from all the huts both before and after the removal, and the results are also shown diagrammatically in fig. 4.

After the removal of the village, the mosquito population attracted to the Experimental Huts was abnormally high for five days and reached $2\frac{1}{2}$ to 6 times the normal proportion of the whole population. After this initial period the distribution remained fairly constant, with the Experimental Catch from the two remaining huts approximately 25 per cent. to 50 per cent. greater in proportion than before the village removal.

The mosquito population in the Inner Control Hut was more affected by the removal than that in the Experimental Huts. It was abnormally high on the second, third and fifth days and then settled down to 100 per cent. to 150 per cent. greater in proportion than before the village removal.

The preliminary survey showed that, before the village was removed, the Experimental Huts attracted less than 10 per cent., and probably less than 5 per cent., of the total Anopheline population in the village. If the same proportion of the local Anopheline population had arrived at the site of the village after its removal the Experimental Catch would probably have increased tenfold whereas it was only 25 per cent. to 50 per cent. greater than before. This indicates that most of the mosquitos had been attracted to the village from a distance, and not merely found their way to its vicinity by chance. This conclusion presents a parallel, on a larger scale, to the conclusion already drawn from the experiments on the relative attractiveness of one and three individuals, in that the number of Anophelines attracted tended to be proportional to the size of the human population.

TABLE II.

Catches of *A. funestus* before and after village removal.

| Date | Before removal Jan. 12–18 | After removal | | | | | | | Jan. 19–25 | Jan. 26–Feb. 2 | Feb. 3–9 |
		19	20	21	22	23	24	25			
No. daily catches	7	1	1	1	1	1	1	1	7	8	7
Experimental Catch (3 rooms)	458	137	285	193	150	135	120	84	1,104	598	651
Inner Control Catch (1 room)	114	24	54	59	29	78	97	23	364	217	338
Outer Control Catch (3 rooms)	385	44	51	27	53	41	102	45	363	323	442
Room average											
Experimental/Outer Control	119%	312	560	710	283	330	118	187	304	185	148%
Inner Control/Outer Control	89%	164	318	650	107	570	284	154	300	202	230%
Room average, Jan. 12–18 = 100%											
Experimental/Outer Control	100%	262	470	600	238	278	99	157	256	156	125%
Inner Control/Outer Control	100%	184	358	730	120	640	320	172	338	226	260%

E2

The marked temporary increases in both the Experimental and Inner Control Catches immediately after village removal, and especially on the second, third and fifth days, are most intriguing. They seem too great to be fortuitous. Could they be due to the still lingering scent of the erased village, or to the return of old mosquitos to " known " localities? If further examples of the effect on Anopheline populations of shifting human populations could be obtained under varying conditions, they might add considerably to our knowledge of Anopheline behaviour.
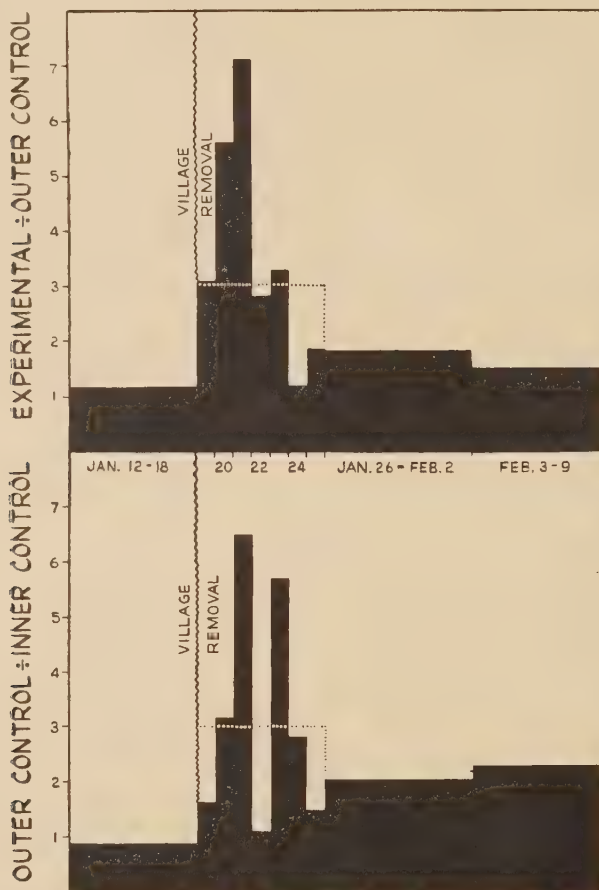
Fig. 4.—Effect of village removal on the proportions of *A. funestus* caught in the Experimental and the Control Huts.

Practical Applications.

No evidence has yet been adduced to show that the number of human hosts is normally an important limiting factor in the determination of the size of an Anopheline population, and it may be supposed that the total number of Anophelines will be more closely related to the capacity of their breeding places than to the number of their human hosts. This factor will help to decrease the malaria risk in crowded communities, and it was mainly for this reason that in an article on camp-siting (Ribbands, 1944b) it was suggested that the number of Anophelines *per*

person in a community tends to be in inverse ratio to the size of that community, so that malaria risk tends to be greater in smaller communities.

The present experiments provide additional support for this view. The village removal, which decreased the size of the community, increased the mosquito infestation by about 50 per cent. in the Experimental Huts and by more than 100 per cent. in the Inner Control Huts. The smaller scale experiment designed to compare the attractiveness of one and three men also showed that despite the acute discrimination of the Anophelines, which led a majority of them to choose the more crowded hut, there were less Anophelines *per person* in this hut, and as Anophelines usually feed without disturbance, and gorge to capacity at one sitting, the malaria risk would tend to be less in the more crowded hut. Evidence that a proportion of Anophelines enter in the first place for shelter, and afterwards feed, reinforces this conclusion.

It is obvious that malaria risks can be reduced or eliminated by extreme isolation, but it has been less generally recognised that in the absence of such isolation the opposite principle holds, and that there is " safety in numbers ".

Summary.

Many female Anophelines wander into huts at random, possibly for shelter, and afterwards feed upon any suitable inhabitants.

This random wandering can be eliminated by pyrethrum spraying, and the acute discrimination of those Anophelines which enter primarily for food can then be demonstrated.

In sprayed huts there was a close relation between the number of men sleeping and the number of Anophelines attracted. The proportions attracted by 3 men and by 1 man were, for *A. melas*, 2·72 ; 1 : *A. gambiae*, 2·38 ; 1 : *A. funestus*, 2·28 ; 1. Discrimination was reduced at one period by the masking odour of cut and withering bush.

There were marked fluctuations in the attractiveness of individuals to Anophelines. On several occasions one or other of the men used as bait suddenly became much more attractive than his fellows, and remained so for several days.

The removal of a village increased by only 25–50 per cent. the proportion of mosquitos caught in two huts which were allowed to remain on the village site. This result indicates that most of the mosquitos which had infested the village had been attracted from a distance by the scent, and had not merely arrived in the vicinity of the village by chance.

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STUDIES ON THE TOXICITY OF INSECTICIDE FILMS*.
II.—EFFECT OF TEMPERATURE ON THE TOXICITY OF DDT FILMS.

By S. PRADHAN.

Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts.

(PLATES III & IV.)

Recent work of Potter and Gillham (1946), as well as other works cited by them, show that temperature has an important effect on mortality due to insecticides. But different effects have been reported in different cases. In the case of contact and stomach poisons the finding in general is that there is higher mortality at lower temperatures but in the case of fumigants the mortality is reported to be higher at higher temperatures. Consequently it was considered desirable to study the effect of temperature on the toxic action of films and preliminary experiments having given unexpected results, interest in the problem was enhanced. A series of more elaborate experiments was, therefore, carried out with the result that probably the whole picture of the temperature-effect may be seen from a new angle. These experiments are described below in chronological order and show how this new conception developed. The techniques followed in these experiments were essentially the same as described in Part I (Pradhan, 1949).

Effect of Temperature on Toxicity of DDT Films to *Tribolium castaneum* Adults.

Experiment I.

Two environments with different temperatures available at the same time were tried : (*a*) a constant temperature cabinet (C.T. cabinet) maintained at 80°F. and (*b*) a basement room wherein the temperature remained between 56–58°F. As humidity was not supposed to have an important effect, no effort was made in the initial stages to record or control humidity.

> 1/11/46. Six filter papers were sprayed with each concentration in 10 per cent. benzene in water with 0·05 per cent. C.H.D.S.† ; time—3–4 p.m. ; weight of deposit in a dish of 9 cm. diameter—0·563–0·573 gms. ; pressure of spray—19 cm. ; temperature during spraying period 67–70°F. ; R.H. 52–51 per cent. After spraying the films were kept in C.T. cabinet to dry overnight.

> 2/11/46. Fifteen insects were enclosed on each film in truncated glass cones and three films of each concentration were kept in the basement room, and three in C.T. cabinet.

> 5/11/46. The inspections were made on a warm C.T. plate.

TABLE I.

Experiment I.—Effect of environment (temperature) on toxicity of DDT films to *T. castaneum* adults.

Concentration gm./100 ccs.			% D		% D+M		% D+M+B	
			80°F.	56–58°F.	80°F.	56–58°F.	80°F.	56–58°F.
Control ...	...	...	0	0	0	0	0	0
0·088	...	...	4·4	6·7	17·7	13·4	22·2	24·5
0·132	...	...	15·9	2·1	29·5	12·4	47·7	27·6
0·197	...	...	18·5*	2·2	25·9*	12·3	40·7*	16·7
0·296	...	...	12·8	6·8	23·5	20·4	49	36·3
0·444	...	...	22·2	11·4	46·6	22·8	66·6	56·9
0·666	...	...	22·2	14·3	55·5	30·9	84·4	80·9
1·000	...	...	34·8	15·2	71·8	32·5	100	100

*27 insects used on this test and not the usual approximately 45.
D=dead   M=moribund   B=badly affected.
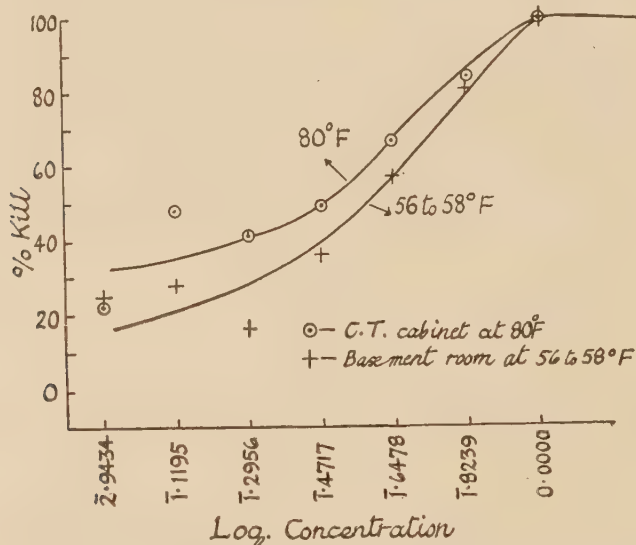
Fig. 1.—Effect of environment (temperature) on toxicity of DDT films to *T. castaneum* (adults).

The results are given in Table I and fig. 1. These data showed, on the whole, less toxic effect in the basement room (56–58°F.) than in the C.T. cabinet (80°F.). They appeared to be diametrically opposed to those of Potter and Gillham (1946) who observed higher mortalities at lower temperatures by spraying the same insect (*T. castaneum*) with the same insecticide (DDT). The difference between the direct spraying technique and the film technique was not considered to be great enough for such a difference in temperature effects. Hence the experiment was repeated.

Experiment II.

Fresh stock solutions and dilutions were used in this experiment.

9/11/46.   Spraying between 11 a.m. and 12.30 p.m. ; films kept in C.T. cabinet for drying.

10/11/46.   Insects enclosed on the film between 11 a.m. and 12.30 p.m. and kept under the two environments as in the first experiment.

13/11/46.   Inspection was carried out on warm C.T. plate.

TABLE II.

Experiment II.—Effect of environment (temperature) on toxicity of DDT films to *T. castaneum* adults.

Concentration gm./100 ccs.			% D		% D+M		% D+M+B	
			80°F.	56–58°F.	80°F.	56–58°F.	80°F.	56–58°F.
Control ...	...	...	0	0	0	0	0	0
0·039	...	...	0	0	4·5	0	9	0
0·058	...	...	6·6	0	8·8	0	16	2
0·088	...	...	8·8	0	13·3	2·2	20	2
0·132	...	...	4·2	0	25·5	0	47	2
0·197	...	...	17·8	0	37·8	2	56	7
0·296	...	...	8·8	2	24·4	2	56	7
0·444	...	...	19·6	4·4	65·2	20	93	69
0·666	...	...	17·8	15·2	55·6	26·1	96	100
1·000	...	...	35·6	6·7	57·8	35·5	98	98

Approximately 45 insects were used for each test.
D=dead   M=moribund   B=badly affected.
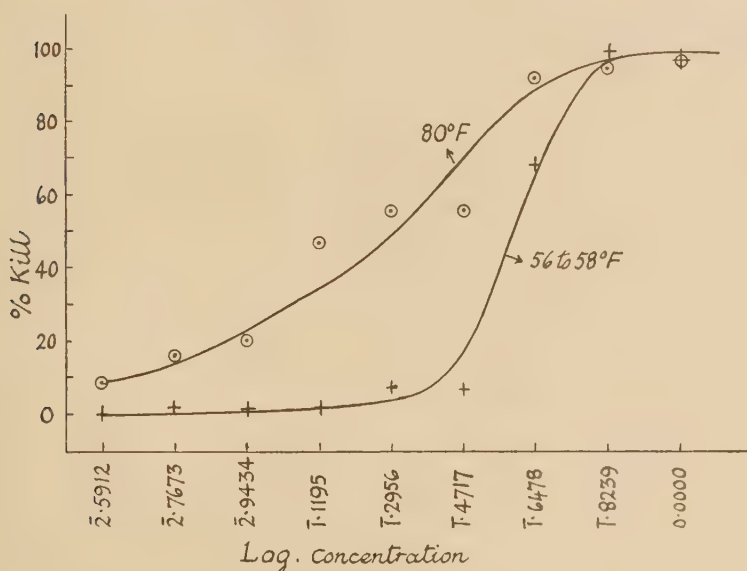
Fig. 2.—The same effect as in fig. 1, symbols also the same.

The results are given in Table II and fig. 2. This second experiment confirmed the results of the first but the batches of insects kept in the basement showed much less toxic effect during this second experiment than during the first. This anomalous difference between the two experiments conducted in apparently the same environment was disturbing and it was decided to carry out further experiments in greater detail.

Experiment III.

The effects of two environments (C.T. cabinet and basement room) on the progress of the toxic effect with time were investigated. Sixty films were made of each of two

concentrations : 0·13 per cent. and 0·296 per cent. DDT emulsion. After drying at the same temperature 30 were kept in the basement and 30 in the C.T. cabinet and insects (*T. castaneum*) were enclosed in glass cones over all of them. For each observation three random films were taken from the C.T. cabinet and three at random from the basement room. Thus 10 observations were made at intervals of a few hours. It was not practicable to conduct the experiment with more than two concentrations.

2/12/46.   Spraying took place between 2 and 5 p.m. ; temperature 70–72°F. ; R.H. 55–50 per cent. ; pressure 19 cm.

3/12/46.   30 dishes of concentration 0·13 per cent. DDT and 30 of concentration 0·296 per cent. in benzene-water-emulsion with 0·05 per cent. C.H.D.S. were removed from the C.T. cabinet to the basement room and 15 insects were enclosed on each of 120 films.   This operation was carried out between 9 and 10 a.m.

The inspections were made by the warm plate technique. The data are given in Table III and fig. 3 (higher concentration). The lower concentration showed a similar effect. The results of the previous experiments were confirmed so far as the effects of the two environments were concerned. On both the concentrations there was a quicker effect in the C.T. cabinet (80°F.) than in the basement room (56–58°F.). But again there appeared to be a disturbing factor in the basement, in as much as the higher concentration did not consistently show a higher toxic effect. Further exploratory tests were undertaken.

TABLE III.

Experiment III.—Effect of environment (temperature) on the progress of toxicity of DDT films to *T. castaneum* adults.

Contact period in hours	Concentration	% D		% D+M		% D+M+B	
		80°F.	56–58°F.	80°F.	56–58°F.	80°F.	56–58°F.
12 ...		0	0	0	0	0	0
25 ...		0	0	0	0	4·4	0
35 ...		0	0	0	0	2·5	0
49 ...		0	0	4·4	0	15·6	0*
58 ...	A	6·5	0*	6·5	0*	34·8	13·3
74 ...		17·8	0	26·7	0	55·6	24·4
82 ...		6·5	0	13·0	0	63·0	21·4
99 ...		34·1	2·2	45·5	4·4	86·4	40·9
106 ...		41·9	4·4	48·9	13·5	95·3	50·0
124 ...		39·5	6·7	44·2	13·4	100	75·6
12 ...		0	0	2·2	0	2·2	0
24 ...		15·9	0	20·5	0	31·8	0
35 ...		0	11·1	2·2	11·1	4·4	15·6
48 ...		0	0	4·4	2·3	6·7	2·3
57 ...	B	9·5	0	11·9	0	33·3	11·4
73 ...		8·5	0	14·9	0	57·4	27·3
81 ...		13·4	2·3	24·4	4·6	77·8	31·8
98 ...		37·2	0	39·5	2·2	83·7	37·8
105 ...		24·4	4·5	35·6	9·0	86·7	61·4
123 ...		44·2	3·4†	53·5	10·3†	93·0	72·4†

D=dead   M=moribund   B=badly affected.

Concentrations :   A=0·2963%   B=0·1317% DDT   *Based on 15 insects.

†On 29 insects, otherwise approximately 45.
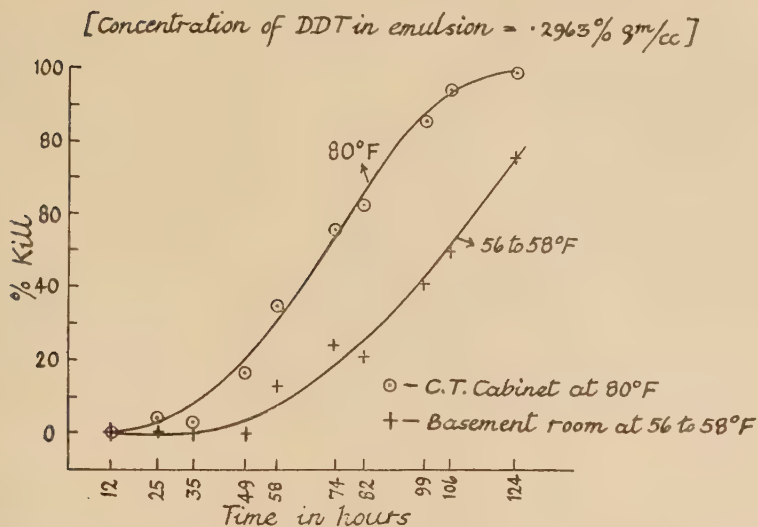
Fig. 3.—Effect of environment (temperature) on the speed of toxicity of DDT films on
T. castaneum adults.

In order to decide whether the difference in toxic effect was due to any change in
the film, the films kept in the basement during the third experiment were transferred
to the C.T. cabinet and those kept in the C.T. cabinet were taken to the basement
room and fresh insects were enclosed on these films on 6th December, 1946, and
inspected on 10th December, 1946. The following proportions were found dead,
moribund or badly affected.

Conc. of spray used to form film	Environment	
	C.T. cabinet (80°F.)	· Basement room (56–58°F.)
0·1317 per cent. ...	12/44	1/44
0·296 per cent. ...	20/47	4/44

Thus the films did not appear to be responsible for the difference in toxic effects
in the two environments.

It was considered possible that the reason for the difference between the results
of the foregoing three experiments and those of Potter and Gillham on direct spraying
might be in the degree of activity of the insect on the film. Fresh insects were there-
fore enclosed at 8 p.m. on 6th December, 1946, on six films of each of the two
concentrations (0·13 and 0·3 per cent.) at 80°F. and the next day at 2 p.m. the
insects from each film were transferred to clean petri dishes and three lots from each
concentration were kept in the C.T. cabinet at 80°F. and the other three in the base-
ment. These insects were examined on 10th December, 1946. This treatment meant
18 hours exposure to the films at 80°F. and about 72 hours separately under two
different environments. The following reactions were observed.

Conc. of spray used to form film	Environment	
	C.T. cabinet at 80°F.	Basement room at 56–58°F.
0·13 per cent.	All unaffected (46) ·	19 out of 43 dead, moribund or badly affected
0·3 per cent.	All unaffected (44)	37 out of 44 dead, moribund or badly affected

The result of this test was now in line with those of Potter and Gillham.

A parallel repetition of the above test was also started at 3 p.m. on 7th December, 1946, when fresh insects were enclosed on another set of films exactly as above but in this case they were removed to clean petri dishes the same day at 8 p.m., *i.e.,* after 5 hours' exposure only. The object of this was that it was thought the 18 hours' exposure of the last test might prove to be too much. These insects were also examined on 10th December, 1946, and gave the following results.

Conc. of spray used to form film	Environment	
	C.T. cabinet at 80°F.	Basement room at 56–58°F.
0·13 per cent.	All unaffected (46)	1 dead, 2 badly affected out of 47
0·3 per cent.	All unaffected (44)	1 dead and 7 badly affected out of 44

At 12.30 p.m. on 11th December, 1946, fresh insects were enclosed on another set of six films of each concentration. All were kept in the basement for 24 hours. On 12th December, 1946, at 12.30 these insects were transferred to clean petri dishes and half were kept in the basement and half in the C.T. cabinet. These insects were examined on 15th December, 1946, between 12.30 and 1.30 p.m. with the following results.

Conc. spray used to form film	Environment	
	C.T. cabinet at 80°F.	Basement room at 56–58°F.
0·13 per cent.	All unaffected (45)	2 out of 45 badly affected
0·3 per cent.	All unaffected (52)	4 out of 46 badly affected

These tests suggested that in the temperature effect on toxic reaction the factor of " pick-up " should be separated from the resistance factor. Hence it was decided to carry out more complete experiments at several concentrations on somewhat similar lines.

At this stage it also became necessary to know the fate of badly affected insects if kept away from the film. This was necessary, because the number of actually dead insects was found to be too small for statistical examination, if observations were made within two or three days. Also precise separation into categories of dead, moribund and badly affected proved too time-consuming in large experiments. For this purpose 27 lots, consisting of 123 badly affected insects from the third experiment, were isolated and kept separately in tubes at 80°F. and examined later. Only two recovered. This was taken to indicate that badly affected insects could be taken to have reached an irreversible stage. Hence it was decided to concentrate mainly on the separation of badly affected insects from those slightly affected, and to add up dead, moribund and badly affected insects when expressing mortality percentage.

Experiment IV.

In this experiment two temperatures (95°F. and 70°F.) were tried. The temperature of the C.T. cabinet was raised to 95°F. and for 70°F. a gas incubator was set up in the basement room.

18/12/46. Emulsions of the same 10 concentrations as in the first experiment were prepared and 6 filter papers were sprayed with each concentration. After spraying the films were kept in the C.T. cabinet to dry. Unlike the previous experiments this time the temperature of the C.T. cabinet was 95°F. instead of 80°F.

20/12/46. About 15 insects were enclosed on each film in a glass cone and three films of each concentration were kept in the C.T. cabinet at 95°F. and the other three of each concentration were kept in the gas incubator at 70°F.

23/12/46. Inspection was carried out by the warm plate technique.

TABLE IV.

Experiment IV.—Effect of environment (temperature) on toxicity of DDT films to *T. castaneum* adults.

Concentration gm./100 ccs.			%D		%D+M		%D+M+B	
			95°F.	70°F.	95°F.	70°F.	95°F.	70°F.
Control	...	...	...	0*	0	0*	0	0*	0
0·039	...	...	...	0	0	0	0	0	76·1
0·058	...	...	...	0	2·2	0	2·2	26·7	75·6
0·088	...	...	...	6·7	4·4	6·7	11·1	13·3	66·6
0·132	...	...	...	44·7	2·2	46·8	2·2	68·1	53·3
0·197	...	...	...	42	0	46	0	74	60
0·296	...	...	...	46·7	8·5	51·1	10·6	84·4	85·1
0·444	...	...	...	52·3	11·1	54·5	17·8	86·4	95·6
0·666	...	...	...	79·5	13·0	81·8	17·4	97·7	93·5
1·000	...	...	...	91·1	10·9	91·1	10·9	97·8	100

Approximately 45 insects used for each test.

* Based on 30 insects.   D = dead   M = moribund   B = badly affected.
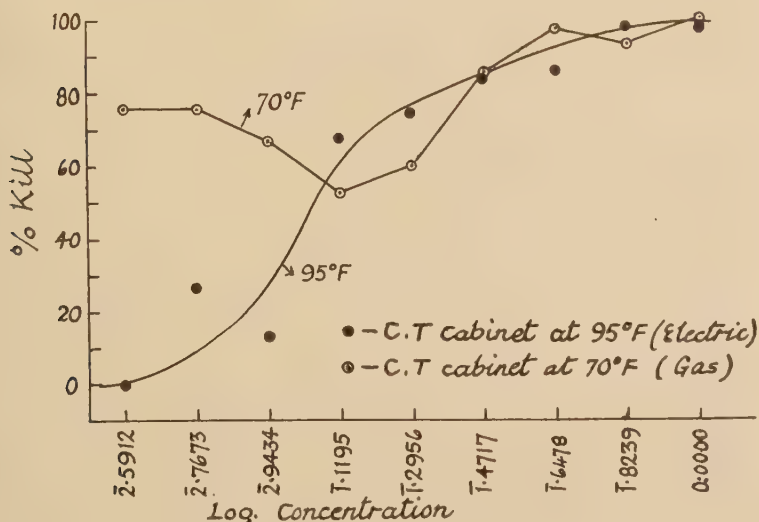
Fig. 4.—Effect of environment on toxicity of DDT films to *T. castaneum* adults.

The results are given in Table IV and fig. 4. This table shows that the percentage values of dead and moribund insects are distinctly higher at 95°F. than at 70°F. but this difference is obliterated when the badly affected insects are also taken into consideration. As the badly affected insects do not survive it could not be concluded from this experiment that the mortality was higher at 95°F. than at 70°F. The high results obtained at 70°F. indicated that some other factor was not being taken into account.

Experiment V.

In this experiment four different temperatures were tried simultaneously in (*a*) an electric incubator maintained at 90°F., (*b*) the C.T. cabinet at 80°F., (*c*) the gas incubator at 70°F. and (*d*) the basement room for the lowest temperature (56–58°F.). After preparing the films the rest of the experiment was divided into two parts as it was impossible to finish all concentrations on the same day.

30/12/46. Spraying was carried out from 3 to 6 p.m. when temperature and humidity were 68°F. and 55 per cent. respectively. The pressure of spray was 18 cm. Twelve filter papers were sprayed with each concentration.

31/12/46. Insects were enclosed on films of lowest six concentrations (S and 1 to 5). About 15 insects were enclosed on each film. Three films of each concentration were kept at each temperature.

1/1/47. Insects were enclosed on the films of last 4 concentrations (6 to 9).

3/1/47. Concentrations 1 to 5 were inspected on warm plate.

4/1/47. Concentrations 6 to 9 were inspected and last of all (S), the sprayed control, was inspected.

The results of the inspection are given in fig. 5 in which the log concentrations are plotted against percentages of badly affected, moribund and dead. It will be noted that the values at 70°F. are higher than those at 80°F. Since the badly affected eventually die of the effect of the poison, this is probably the best plot, but it should be stated that taking into account only the moribund and dead, although there is some irregularity in the figures at 70°F., toxicities run 90° > 80° > 70° > 56°.

TABLE V.

Experiment V.—Effect of environment (four temperatures) on toxicity of DDT films to *T. castaneum* adults.

Concen-tration gm./100 ccs.	%D				%D+M				%D+M+B			
	A 90°F.	B 80°F.	F 70°F.	D 56 to 58°F.	A 90°F.	B 80°F.	F 70°F.	D 56 to 58°F.	A 90°F.	B 80°F.	F 70°F.	D 56 to 58°F.
Control	—	—	—	—	—	—	—	0	—	—	—	0
0·039 ...	4·6	0	0	0	4·6	0·3	0	0	14·4	7·4	45·6	0
0·058 ...	13·0	0·2	0	0	23·8	0·2	0	0	34·0	11·2	51·0	0
0·088 ...	20·4	2·3	0	0	25·1	8·8	0	0	54·9	37·0	52·3	2·2
0·132 ...	57·8	6·8	0·3	0	71·1	6·8	0·3	0	93·1	42·3	61·1	10·6
0·197 ...	86·4	4·8	0·1	0	88·7	4·8	0·1	2·2	95·3	50·1	71·1	6·5
0·296 ...	80·9	12·9	2·5	0	82·9	21·3	37·3	0	93·4	46·8	81·4	15·9
0·444 ...	82·9	4·8	0	0	82·9	13·9	2·3	7	94·9	59·2	81·8	14·0
0·666 ...	95·6	20·1	2·2	2·1	95·6	33·3	2·2	6·4	100	97·8	97·9	89·4
1·000 ...	100	61·4	2·5	2·1	100	65·9	9·5	2·1	100	100	100	100

The experimental details regarding surface, medium, etc., were the same as in Experiment **I**. Approximately 45 insects used for each test.

D = dead   M = moribund   B = badly affected.

It was clear from all these five experiments that although the importance of temperature was clearly revealed in its effect upon potency, there was some disturbing factor which tended to modify the results to different degrees in each experiment. The humidity factor was regarded to be of practically no significance yet the four different environments showed no essential differences except in temperature and humidity. The relative humidity was therefore determined in each of the four environments. It was found to be about 20 per cent. in the electric incubator at 90°F., 51 per cent. in C.T. cabinet at 80°F., about 80 per cent. in the gas incubator at 70°F. and about 50 per cent. at 56°–58°F. in the basement. It was remarkable that the relative humidity was 80 per cent. in the gas incubator whereas in the basement room, in which it stood, the humidity was about 50 per cent. but it was confirmed by several tests. Probably a leak of water vapour from the surrounding water jacket of the gas incubator was responsible. Under these circumstances it was decided to carry out a small preliminary test on the effect of humidity at different temperatures.
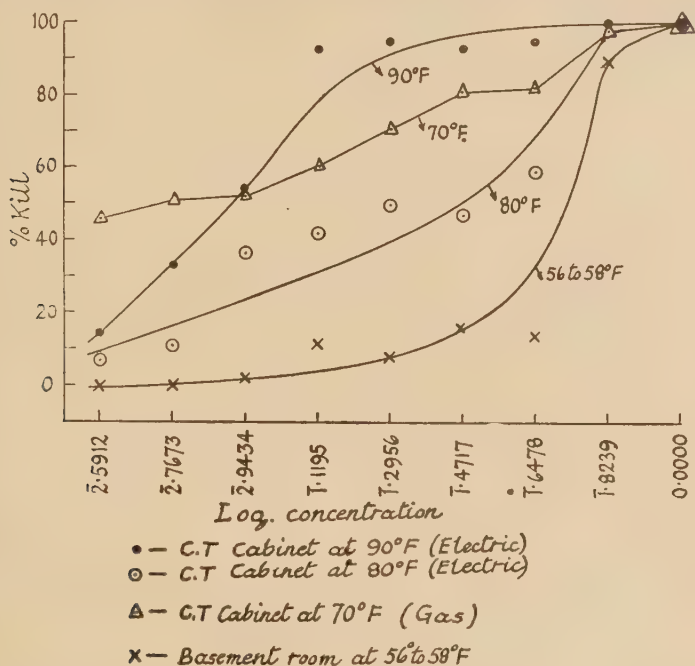
Fig. 5.—Effect of environment on toxicity of DDT films to *T. castaneum*.

The result of this test was that at the higher humidity of about 84 per cent. there was shown a considerably higher toxic effect. It was clear that the relative humidity in addition to temperature would have to be taken into account in the assessment of the toxic effect of insecticide films.

Experiment VI.

The tests described above (p. 244) suggested the possibility of separating the factor of "picking-up" from that of resistance. It was decided therefore to follow up this approach with due consideration for the effects of humidity. In experiment VI, therefore, the insects were kept in contact with the film at 80°F. for about 24 hours and then transferred to clean petri dishes, divided into four lots and kept at four different temperatures in separate humidity chambers, specially improvised for the purpose which will be described in Part III.

17/1/47.   Emulsions of the same 10 concentrations (S and 1 to 9) as in experiment I were prepared and 12 filter papers were sprayed with each concentration, and kept in the C.T. cabinet at 80°F. for drying.

18/1/47.   Insects were enclosed (11.30 a.m. to 12.30 p.m.) on the 6 lowest concentrations (Control S and 1 to 5) and all the films of these concentrations were kept in the C.T. cabinet at 80°F.   Glass cones were used for confining the insects.

19/1/47.   Insects were enclosed over the remaining 4 concentrations (6 to 9) as on 18/1/47 and kept at 80°F.

The insects enclosed on S and 1 to 5 concentrations were transferred to clean glass surfaces in petri dish humidity-chambers with sodium bichromate solution to ensure more or less constant humidity (50 per cent.).   Insects from three films of each concentration were kept at each of the 4 temperatures.

24/1/47.   Insects enclosed on the films (6 to 9) on 19/1/47 were transferred to clean glass surfaces in humidity chambers and kept at 4 different temperatures.

22/1/47.   Insects exposed to concentrations 1 to 5 were inspected on warm plate.

23/1/47.   Insects exposed to concentrations (6 to 9) were inspected on warm plate.

The results are given in Table VI and fig. 6.   They show a higher toxic effect in insects kept at temperatures lower than 80°F.; but at 90°F. the toxic effect was slightly higher than at 80°F. except at the two highest concentrations.

TABLE VI.

Experiment VI.—Effect of temperature on toxicity of DDT films to *T. castaneum* adults: contact at *one* temperature (80°F.) and reaction at *four* temperatures.   Relative humidity about 50 per cent.

Concentration gm./100 ccs.				Per cent. (dead, moribund and badly affected)			
				A 90°F.	B 80°F.	C 70°F.	D 56–58°F.
Control S	...	...	...	0	0	0	0
0·039	...	...	...	16·7	6·4	0	40·9
0·058	...	...	...	25	7	25·6	69·4
0·088	...	...	...	15·9	0	25·6	88·5†
0·132	...	...	...	15·9	9·3	47·5	93·0
0·197	...	...	...	23·4	8·5	54·3	74·5
0·296	...	...	...	7·8	2·2	81·6	95·7
0·444	...	...	...	32	14·9	93·2	100
0·666	...	...	...	61·2	91·8	100	84·8
1·000	...	...	...	95·7	97·6	100*	100

The number of insects used for each experiment was 40–50.
*37 insects were used.   †61 insects were used.

Experiment VII.

In this experiment insects were kept in contact with the film in separate humidity chambers at 4 different temperatures for about 24 hours, then transferred to glass surfaces in the same humidity chambers and kept at the same temperature (70°F.) till inspection.   The same films were used as in experiment VI.

24/1/47.   The films used in experiment VI were put in humidity chambers (about 50 per cent. R.H. maintained by a saturated solution of sodium bichromate).   Between 11 a.m. and 6 p.m. insects were enclosed on the films of the 8 lowest concentrations, including the sprayed control S and 1 to 7.

25/1/47.   Between 12 noon and 5 p.m. all the insects were transferred to the glass surface in each humidity chamber.   The insects were removed from the film in the same order (starting with the lowest concentration) in which they were enclosed.   This ensured an almost equal period of contact with the film in case of each replication. After this operation all the insects were kept at 70°F. for reaction.

28/1/47.   Inspection was made on the warm plate following again the same order in which the insects were enclosed on the film.

The results are shown in Table VII and fig. 7.   In this experiment the insects kept in contact with the films at higher temperatures showed much more toxic effect than those kept on the films at lower temperatures.   The results are fairly clear cut. In fact, the effects are so far apart that it is difficult to apply the ordinary probit test because at 90°F. there has been 100 per cent. mortality at all concentrations except one and at 56 to 58°F. there has been zero per cent. mortality at all except two concentrations.
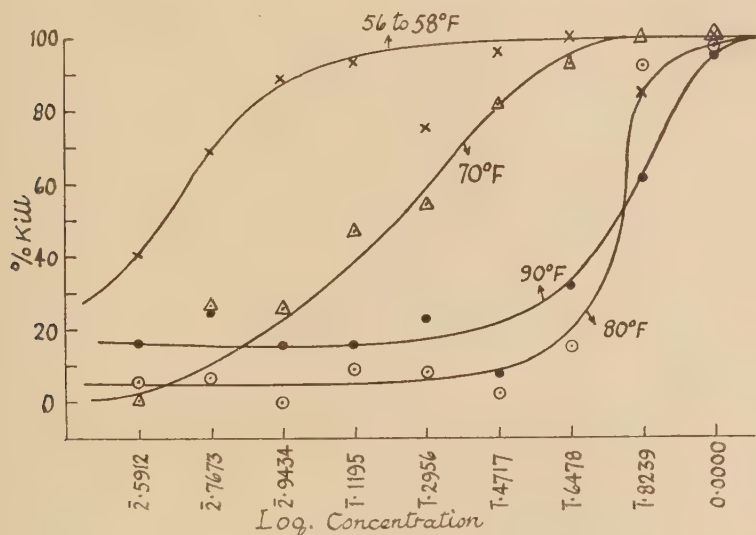
Fig. 6.—Effect of temperature on toxicity of DDT films to *T. castaneum* adults : contact at *one* temperature (80°F.) and reaction at *four* temperatures.

TABLE VII.

Experiment VII.—Effect of temperature on toxicity of DDT films to *T. castaneum* adults: contact at *four* temperatures and reaction at *one* temperature.   Relative humidity about 50 per cent.

Concentration gm./100 ccs.				Per cent. (dead, moribund and badly affected)			
				A 90°F.	B 80°F.	F 70°F.	D 56–58°F.
Control S	...	...	...	0	0	0	0
0·039	...	...	...	65·8	2·2	0·1	3·4*
0·058	...	...	...	100	24·4	0	0
0·088	...	...	...	100	13·6	0	0
0·132	...	...	...	100	18·2	0	0
0·197	...	...	...	100	31·0	6·6	0
0·296	...	...	...	100	37	6·6	4·4
0·444	...	...	...	100	73·3	46·6	34·9

The same films were used as in Experiment VI.

Approximately 45 insects were used for each test.   *Based on 29 insects.
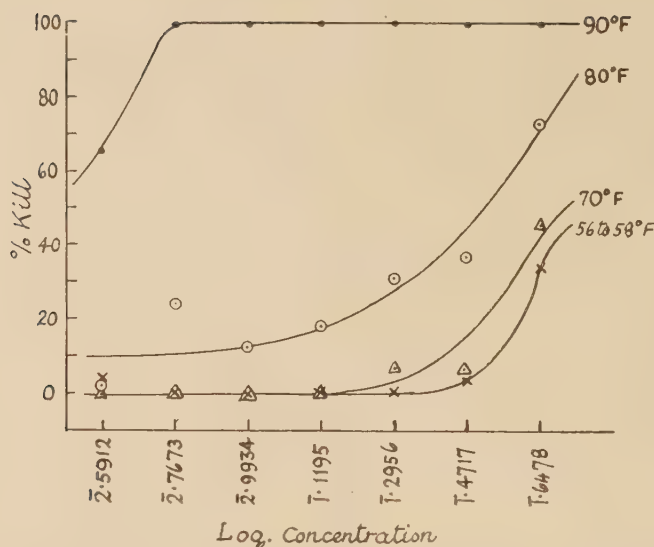
Fig. 7.—Effect of temperature on toxicity of DDT films to *T. castaneum* adults: contact at *four* temperatures and reaction at *one* temperature.

Experiment VIII.

This was a final repetition of experiment V making use of the constant humidity chambers to offset the effect of humidity—if it was really the disturbing factor in the first five experiments.

3/2/47. Emulsions of the same 10 concentrations (S and 1 to 9) as in the first experiment were prepared and 12 filter papers were sprayed with each concentration. After spraying, the filter papers were kept in the C.T. cabinet at 80°F. to dry.

4/2/47. The films were kept in the humidity chambers (about 50 per cent. R.H. maintained with saturated solution of sodium bichromate).

5/2/47. Insects enclosed on films of lowest six concentrations (S and 1 to 5) between 8.30 a.m. and 1 p.m.

6/2/47. Insects enclosed on films of concentrations 6 to 9 between 12 and 4 p.m.

8/2/47. Inspection on warm plate of insects confined on films of concentrations 1 to 5.

9/2/47. Inspection on warm plate of insects confined on films of concentrations 6 to 9 and S.

TABLE VIII.

Experiment VIII.—Effect of temperature on toxicity of DDT films to *T. castaneum* adults: continuous contact at *four* temperatures.   Relative humidity about 50 per cent.

Concentration gm./100 ccs.	Per cent. (dead and moribund and badly affected)			
	A 90°F.	B 80°F.	C 70°F.	D 56–58°F.
Control S	0	0*	0	0
0·039	27·3	2	10·7	0
0·058	79	27·7	12·6	0
0·088	95·5	22·9	14	0
0·132	84·4	32·7	18·8	0
0·197	91·4	24	34·7	2
0·296	79·5	39·2	32	2
0·444	98	92·3	98·1	47·2
0·666	100	92·9	100	90·9
1·000	100	100	100	93·3

Details regarding surface, medium, etc., were the same as in Experiment VI.
45–50 insects used in each test.   * Based on 38 insects.
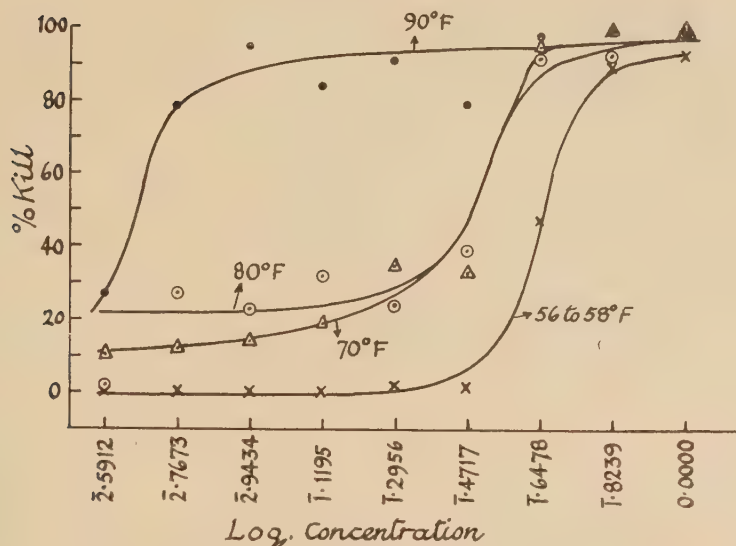
Fig. 8.—Effect of temperature on toxicity of DDT films to *T. castaneum* adults : *continuous* contact at *four* temperatures.

The results are given in Table VIII and fig. 8. They show a definitely higher toxic effect at 90°F. and a definitely lower toxic effect at the lowest temperature (56 to 58°F.), the effects at 80 and 70°F. being intermediate between the two. On four out of nine concentrations the effect at 80°F. is higher than at 70°F., on four higher at 70°, whilst on one the mortality was 100 per cent. at both temperatures.

Conclusions.

The last three experiments reveal the following facts, and those prior to them give general support to the conclusions drawn from them.

(1) When kept in contact with the film for a short time at the same temperature and then removed to a clean surface at different temperatures, there is, in general, a lower mortality at the higher temperature. The sixth experiment showed the following order of toxic effect with temperature : 56–58 > 70 > 80 = or < 90°F. (difference between 80 and 90° not significant).

(2) When insects are kept in contact with the film for a short time at different temperatures and then kept on clean surfaces at the same temperature, there is a higher mortality in insects kept in contact with the film at higher temperature. The seventh experiment showed highest mortality in insects kept in contact with the film at 90°F. and it was progressively lower in insects kept in contact with the film at 80°F. and 70°F. and 56 to 58°F.

(3) When insects are continuously kept in contact with the film at the four different temperatures, those at 90°F. give highest mortality, those kept at 56 to 58°F. showed the lowest mortality and mortality values are intermediate in those kept at 80°F. and 70°F. but the difference between 80°F. and 70°F. is uncertain and insignificant.

The above three observed facts, taken together, show that in studies of the toxicity of insecticide films at different temperatures two quite distinct factors have to be kept in view (a) the pick-up of the dose, (b) the basic effect of temperature on the reaction between the insect and the insecticide or the effect of temperature on

the resistance of the insect. Experiment VI giving a higher kill at lower temperatures indicates that like certain other activities of living organisms the resistance of the insect increases with temperature up to a point. Experiment VII on the other hand, giving a higher kill in insects kept in contact with the film at higher temperatures shows that the pick-up of the dose also increases with temperature. Experiment VIII gives the resultant of these two opposing factors (*a*) resistance, and (*b*) pick-up, both of which increase with temperature. In this connection, however, it should be borne in mind that insect activity does not go on increasing indefinitely with temperature. For example, insect activity has a maximum value on the temperature scale and experiment VI shows that probably 90°F. is a little beyond the point conferring maximum resistance.

The investigations outlined above also indicate that as far as the toxic action of films are concerned the effects of humidity can introduce complicating factors. A more detailed study of the importance of humidity in this connection will be given in Part III.

Effect of Temperature on Toxicity of DDT Films to Larvae of *Plutella maculipennis*.

The aim of these investigations was to see how far the conclusions arrived at in the case of *T. castaneum* are applicable in the case of a totally different insect. For this purpose the larva of *Plutella maculipennis* was chosen. This species, however, presented certain specific difficulties. As indicated earlier (Pradhan, 1949) these larvae could not be confined on the film by means of glass cones or cylinders since they are capable of crawling up them and away from the toxic film. Hence they had to be confined within cones of bolting silk having a film on their inner surface and the bottom film also had to be on bolting silk instead of filter paper. This modification, however, did not completely solve the difficulty, because these larvae are in the habit of spinning silk threads which cover the insecticide film and also in extreme cases form a rather dense web under the enclosing cone ; at times a number of these larvae are found suspended in this web away from the insecticide film. The following series of experiments tend to show that this habit appeared to modify the effect of temperature on toxicity to these larvae. The disturbance due to cannibalism has already been referred to (Pradhan, 1949) ; it could be kept within negligible limits by finishing the essential part of each experiment within the first 24 hours of starvation or by so planning the experiment that larvae need not be starved for more than 24 hours.

Experiment IX.

In this experiment the larvae of *Plutella maculipennis* were kept in continuous contact with the DDT film for about 24 hours at two temperatures, 80°F. in a C.T. cabinet and about 58°F. in the basement room. The humidity was kept about 50 per cent. R.H. by carrying on the experiment in constant humidity chambers containing saturated solutions of sodium bichromate.

8/4/47. Benzene-water emulsions of five concentrations of DDT with 0·05 per cent. C.H.D.S. as adjuvant, were prepared as usual and 12 circles of bolting silk were sprayed with each concentration. After spraying, the films were kept in a constant temperature cabinet at 80°C. to dry.

10/4/47. Six bolting silk circles with films of each concentration were converted into cones and 28–30 *Plutella* larvae were enclosed within each of these on the respective films between 2 to 4 p.m. Each replication was kept in a separate petri dish. The C.H. chambers with three of them were kept in the C.T. cabinet at 80°C. and the other three in the basement at about 58°C.

11/4/47. Inspection was carried out between 11 a.m. and 1 p.m. and insects sorted out into the usual five categories.

TABLE IX.

Experiment IX.—Effect of temperature on toxicity of DDT films to larvae of *Plutella maculipennis* : continuous contact at *two* temperatures.

Concentration gm./100 ccs.	Per cent. (dead and moribund and badly affected)	
	80°F.	56–58°F.
Control	33·3	10
0·058	56·7	14·8
0·088	69	20
0·132	96·8	27·6
0·196	86·7	32·1
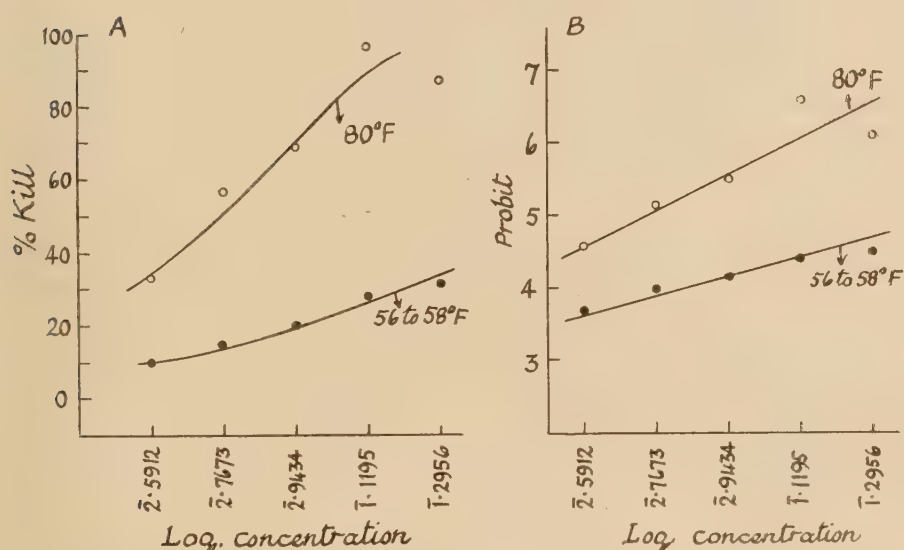
Fig. 9.—Effect of temperature on toxicity of DDT films to larvae of *Plutella maculipennis* : continuous contact at *two* temperatures.

The percentage of insects dead, moribund or badly affected are plotted against concentration in fig. 9. Since the results are closely similar to those obtained in the case of *T. castaneum*, the tentative conclusion was drawn that both *P. maculipennis* and *T. castaneum* react in the same way. It was decided, however, to repeat in this case also, the critical experiments carried out with *T. castaneum*.

It should be noted that if assessment is based on the percentage of insects actually dead or on the percentage of dead and moribund taken together the difference found remains in the same direction.

Experiment X.

In this experiment 12 batches of insects were enclosed on films of each concentration. Six of these batches were kept in contact with the films at 80°F. in C.H. chambers at about 50 per cent. R.H. and the other six in the basement at about 60°F. and in similar C.H. chambers at about 50 per cent. R.H. After 5 hours contact with the films they were transferred to glass surfaces in the same C.H. chambers and

3 from each lot of six batches were kept in a C.T. cabinet at 80°F. and the other three in the basement at about 60°F. Thus this experiment combined the principles of experiments VI and VII with *T. castaneum* (pp. 247–250) and the effect of 4 different treatments could be observed, two contact temperatures and two reaction temperatures.

20/5/47. Five different concentrations of DDT in benzene-water emulsion with C.H.D.S. as adjuvant were prepared and 24 circles of bolting silk were sprayed with each concentration and kept in the C.T. cabinet to dry. The spray pressure was 19 cm., temperature 65°F. and humidity 50 per cent. R.H.

24/5/47. Twelve bolting silk circles with DDT films of each concentration were converted into cones and the other circles were kept for the bottom film. Thus twelve cages for each concentration of DDT film were ready. Each of these cages was put in a C.H. chamber containing saturated solution of sodium bichromate. Between 3 and 5 p.m. 12 batches of 10 larvae each were enclosed on the film of each concentration and six batches were kept in the C.T. cabinet at 80°F. and the other six were kept in the basement at about 60°F.

Between 7 and 9 p.m. the larvae of each batch were transferred to a glass surface in the same C.H. chambers and enclosed with a piece of cabbage leaf within glass rings with a top of perforated zinc plate. Out of the six batches kept in C.T. cabinet three were again kept in the same cabinet and the other three were kept in the basement. Similarly three from those kept in contact with the film in the basement were transferred to C.T. cabinet and the other three kept back in the basement.

27/5/47. Inspection was carried out as usual. By this time the reaction was quite clear-cut because the majority of the larvae had either died or pupated.

TABLE X.

Experiment X.—Effect of temperature on toxicity of DDT films to larvae of *P. maculipennis* : contact at *two* temperatures and reaction at *two* temperatures.

	Per cent. (dead and moribund and badly affected)			
Contact for 4 hours at ...	80°F.		60°F.	
Reaction for 3 days at ...	80°F.	60°F.	80°F.	60°F.
Concentration gm./100 ccs.	A	B	C	D
Control	(3·3)	(3·3)	(8)	(0)
0·039	0	0·4	3·4	3·8
0·058	0	15·1	0	20·7
0·088	10·8	31	0	60
0·132	8·1	82·7	30·1	90
0·197	3·4	69·3	26·2	93·3

The results are given in Table X and fig. 10 (A and B). It is clear that, irrespective of the temperature at which the larvae were kept in *contact* with the film, those which were kept for *reaction* in the basement at 60°F. showed a much higher susceptibility than those kept in the C.T. cabinet at 80°F. This reaction is identical with that obtained with DDT and *T. castaneum*. But, the difference between those kept in *contact* with the film at two different temperatures and maintained for *reaction* at the same temperature (compare column A with C and B with D) is, in this case, opposite of what was found in the case of *T. castaneum*. The figures in column A are less than those in column C (except at one concentration) and those in column B are less than those in column D. The obvious reason for this peculiar effect appeared to be that the larvae used were at a very advanced stage and quite ready for pupation. At the higher temperature these larvae began to form cocoons soon after they were enclosed on the film and thus the contact with the film was avoided or reduced in duration in

many cases. At the lower temperature on the other hand, the cocoon formation was delayed, and the larvae remained in contact with the film for the full period. This apparently was a special case in which the age of larvae played such a major rôle that the effect of temperature seemed to be reversed. The experiment was therefore repeated with younger larvae. The results of this repetition are given in Table XI. Obviously this experiment confirms the above results that unlike *T. castaneum* the larvae of *P. maculipennis* enclosed on DDT films at 60° for a short period appear to pick up a greater dose of DDT than if they were enclosed on the film at higher temperature (80°F.).
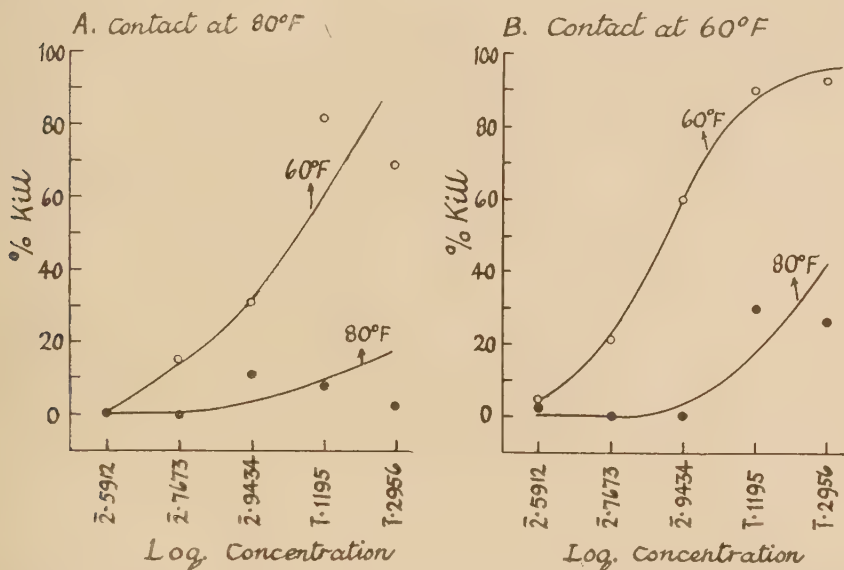
Fig. 10.—Effect of temperature on toxicity of DDT films to larvae of *P. maculipennis*.

TABLE XI.

Effect of temperature on toxicity of DDT films to young larvae of *P. maculipennis* : contact at *two* temperatures (80°F. and 60°F.) and reaction at *one* temperature.

Concentration gm./100 ccs.	Per cent. (dead and moribund and badly affected)	
	80°F.	about 60°F.
Control	0	0
0·039	0	0
0·058	18	4
0·088	17	47
0·132	24	50
0·196	28	67

25–30 insects were used for each test.

Probable cause of greater pick up at lower temperature.

In view of the very striking difference in cocoon formation observed at the temperatures employed in the tenth experiment, it was considered possible that there may be a difference in the amount of silk thread secreted by these larvae at two temperatures, that this silk might cover the DDT film to different degrees at the two temperatures and that this fact may be responsible for higher pick-up at lower temperatures. With this consideration in mind two cages of unsprayed bolting silk were prepared and 10 larvae of *P. maculipennis* were enclosed in each cage kept in the C.H. chamber (at about 50 per cent. R.H.). One of this batch was kept in the C.T. cabinet at 80°F. and the other in the basement at about 60°F. Next day, i.e. after about 18 hours enclosure, the larvae were shaken away and the bolting silk of the cage was examined. The silk on which larvae were enclosed at 80°F. showed a shining spot (Plate IIIa) over the area where larvae were confined. The examination of this spot under a binocular microscope revealed that the mesh of bolting silk was densely covered with silk threads secreted by the larvae. Photomicrographs of the border of this spot are given in Plates IIIb and IVa illustrating both the normal mesh of bolting silk and the silk secreted by the *Plutella* larvae. A similar photomicrograph of the silk on which the larvae were confined at the lower temperature is given in Plate IVb which obviously indicates that much less silk was secreted by the larvae at lower temperatures. Both photomicrographs represent the visibly densest portion of secreted silk on the two pieces of bolting silk. Thus the lower pick-up of DDT at the higher temperature was most probably due to the greater amount of silk covering the DDT film at the higher temperature.

A further experiment was carried out in which very young and more susceptible larvae were subjected to continuous contact with the film at 80°F. and 60°F. The results confirmed those of the first experiment with *P. maculipennis*, the higher temperature showing the greater effect.

Conclusions.

The following conclusions may be drawn from the foregoing experiments :—

(1) When larvae of *P. maculipennis* are kept in continuous contact with DDT films at 80°F. and about 60°F. for about 24 hours, there is a higher mortality at the higher temperatures within this period of time (experiment IX).

(2) When these larvae are kept in contact with DDT films at the same temperatures (80°F. or 60°F.) for 4 or 5 hours only and thereafter transferred to a glass surface and kept at the two different temperatures of reaction, there is higher mortality at the lower temperature of reaction (experiment X).

(3) When these larvae are kept in contact with DDT films at 80°F. and 60°F., for about 4 hours and are thereafter transferred to a glass surface and kept at the same temperature (80°F. or 60°F.), then there is higher mortality in those larvae which were kept in contact with the film at about 60°F. (experiment X).

A comparison of these results with those obtained in the case of *T. castaneum* shows that (1) and (2) are identical in the two cases but that (3) is the reverse of what was observed in the case of *T. castaneum*. The cause of the latter difference appears to be the greater activity of the larvae, the higher temperature resulting in production of greater amounts of silk which covers the DDT film thus probably reducing the dose of DDT picked-up. The difficulty remains, however, that it is not possible to reconcile all the three conclusions in the case of *Plutella* itself, (2) and (3) indicated that both the " pick-up " of DDT as well as the susceptibility of the larvae are higher at the lower temperature. Yet, in the case of continuous contact there is a higher mortality at the higher temperature. It is clear from this anomalous behaviour that some unrecognised factor is playing a part in the effect.

Discussion and Review of the Literature.

A perusal of the literature indicates that probably most of the apparently conflicting observations of various workers can, at least partially, be cleared up if the conclusions drawn from the experiments with *T. castaneum* and DDT films are put in the form of the following three basic generalisations :—

(1) That insect resistance to poisons is affected by temperature in essentially the same manner as all other insect activities, *i.e.* there is an increase of resistance with temperature up to a certain degree and then a decrease with any further rise of temperature.

(2) That the amount of poison reaching the site of action in unit time is also affected by temperature but in various ways depending on circumstances. Generally, this amount increases with temperature but the reverse can also take place. The exact steps through which this increase or decrease is brought about must be complex but insect activity which increases with temperature has obviously much to do with it. Thus for example, increased locomotor activity appears to increase the pick-up of DDT from a film and increased respiratory activity appears to increase the amount of poison fumes entering the tracheae ; on the other hand increased silk spinning activity of *P. maculipennis* larvae appears to decrease the pick-up from DDT film.

(3) The apparent rise or fall in toxicity with increase in temperature is determined by the above two factors, namely, resistance and pick-up.

If the conclusions expressed above are correct, one would expect that irrespective of the form in which the poison is administered, *i.e.* in the case of a contact insecticide in the form of spray, dust, or film, as stomach poison or fumigant, increase in temperature within limits *during* treatment (*i.e.* during exposure or contact) will increase the toxic effect and the increase in temperature within limits *after* treatment (*i.e.* during the major part of reaction) will decrease the toxic effect. In the case of the treatment being a prolonged operation and continuing over the major portion of the reaction time the temperature effect will be the resultant of two opposite factors, resistance and pick-up (or absorption). As regards the temperature before treatment a higher temperature will result in an increase both in resistance as well as activity ; the former decreasing the toxic effect and the latter likely to increase the pick-up. Hence, the resultant effect should be comparatively less and should depend on the duration and method of treatment during which the effect of the previous treatment is expected either to persist or to disappear. Although much more systematic work is needed to understand fully the actual manner in which temperature affects toxicity, it is instructive to note from the following short review that most of the observations published so far, conform to the above expectations.

Contact poison administered as liquid spray.

Nine references were examined regarding the effect of temperature on the toxicity of sprays. Only in two were clear distinctions recognised between the separate effects of temperature during different stages of experimentation, namely (*a*) before treatment, (*b*) during treatment, and (*c*) after treatment. One reports the effects of temperature before and after treatment and the other gives effects of temperature during and after treatment. There appears to be no published account giving the effects of temperature during all three stages. In the rest a careful study of the technique is needed to decide which effect is being described. Nevertheless, the observations reported in seven out of the nine papers examined agree with expectation in the light of the generalisations given above. The techniques used in the other two are not quite clear on this point.

Effects of temperature during and after treatment.

Harries & others (1945), while working on some factors affecting the insecticidal action of pyrethrum extracts on the beet leafhopper (*Eutettix tenellus*, Bak.), studied the effect of the temperature under the following conditions " when the leafhoppers were (1) sprayed with pyrethrum extract in oil at different temperatures and then held for 24 hours at these same temperatures, (2) sprayed at a common temperature and then held at different temperatures, (3) sprayed at different temperatures and all held at 80°F." Quite obviously the plan of experimentation is very similar to that adopted with DDT films and *T. castaneum*. Although these workers were using a quite different technique (direct spraying), a different insecticide (pyrethrum), which, moreover, is subject to rather rapid loss of activity on exposure, and a quite different insect (beet leafhopper) the tabulated results are essentially similar to those observed with DDT films and *T. castaneum* adults. They consistently found higher toxicity with higher temperature during spraying, and with lower temperature after spraying. When, however, the same temperature was maintained during and after the spraying, the resultant effect was, unlike that of DDT films and *T. castaneum*, a higher kill at lower temperature. However, Harries and his colleagues seem to have been concerned only with the practical aspects of these results as they summarise their conclusions thus :—" These tests showed that the mortality of leaf hoppers sprayed with pyrethrum in oil could be increased by raising the temperature at the time of application but could be increased to a greater extent by lowering it after application. As a practical application of these findings it may be inferred that the best results in control would be obtained by treatments made in late afternoon, which would naturally be followed by lower temperatures at night."

Effect of temperature before and after treatment.

Potter and Gillham (1946), on the basis of whose work the present work was started, studied the effect of temperature before and after spraying on the toxicity of various contact poisons to adult *T. castaneum*, Hbst. With four poisons (pyrethrins, pyrethrins and terpineol, lauryl thiocyanate and nicotine) they tested the effect of temperature after spraying and in all cases they found higher toxicity at lower temperatures. With the same materials they found that low temperatures before spraying increased the toxic effect of the first two and slightly decreased that of the last two. On the whole, cooling before treatment was less effective than cooling after treatment. They maintained the same temperature before and after spraying with seven poisons (four mentioned above, 3 : 5 dinitro-*o*-cresol, DDT, and Wakefield half-white oil) and with each except the last there was a higher toxic effect at lower temperatures. The general conclusions of Potter and Gillham also fit fairly well into the present study, for they conclude, " It seems probable that the increase in toxicity under cool conditions of after treatment is due to the physiological conditions of the insect at these temperatures and not to the effect of these temperatures on the poison or the medium in which it is carried. The evidence for this statement is that the increase occurred when chemically stable and chemically unstable, volatile and non-volatile, liquid and solid poisons, and when volatile and non-volatile media were used."

Effect of temperature after treatment only.

Hartzell and Wilcoxon (1932) sprayed a few (30) rose chafer adults (*Macrodactylus subspinosus*, F.) with pyrethrum and after the insects became moribund exposed them in batches to low and high temperatures and observed recovery and death. On the basis of this simple experiment they suggest that " the process of recovery and death are both accelerated at higher temperatures. . . . If the insects have received a dose insufficient to kill, recovery is more rapid, but if, on the other hand, the dose is lethal death occurs more rapidly at the higher temperatures." Although the experimental basis for the conclusion is almost negligible, the suggestion is

certainly worth investigation.   Although no particular experiment to this end was carried out, the general observations made throughout the present investigations have left a similar impression.   Several others have also reported that, although the survival percentage increases, the survival period decreases at higher temperatures. In a review by Potter and Gillham (1946) it is stated that Klinger (1936) found that the toxicity of pyrethrum and derris sprays to a variety of insects varied inversely with after-treatment temperature.   More recently Eagleson (1942) described the effect of temperature on the recovery of houseflies from the knock-down due to the toxic effects of the pyrethrins and lethane (β-butoxy-β-thiocyano-diethylether). He tried five temperatures (22, 26, 30, 34, and 38°C.) with both poisons and in both cases established that percentage of recovery increased regularly with temperature.

Effect of temperature during treatment only.

Fleming (1933) reported that pyrethrum sprays were much more effective against the Japanese beetle (*Popillia japonica*, Newm.) if applied in warm sunny weather than under cool cloudy conditions.   David (1946) while working on factors influencing the interaction of insecticidal mists and flying insects, exposed *Aëdes aegypti* adults to insecticide mists (pyrethrum) at 20° and 30°C. and found that " a 10°C. drop in temperature reduced the observed kill by about 20 percent."   Both these observers ascribe the increased toxicity to increased activity at higher temperatures. Chapman & others (1943), however, found that increase in air temperature from 42 to 78°F. and that of spray fluid from 40 to 70°F. had no significant effect on the insecticidal action of oil sprays on *eggs* of fruit-tree leaf roller (*Archips argyrospila*, Walker).   This absence of temperature effect may be due to lack of movement in eggs.

The observations of Barnes (1946), Whitcomb (1935, 1936) and Böttcher (1938, 1939) are, on the other hand, not easy to reconcile with the views expressed above. Barnes, while studying the effect of temperature on resistance of bed bugs (*Cimex lectularius*, L.) to DDT, sprayed the insects and stored them " for six days at the temperature at which they were originally kept, before mortality counts were made." It was found that the percentage of kills among bugs maintained at 30°C. were " consistently greater than among those kept at 23 and 25°C."   These observations are directly opposed to those of Potter and Gillham (1946) obtained with DDT sprays and *T. castaneum*.   It is difficult, however, to evaluate correctly these results, since it is not clear whether, after spraying, the insects were stored on the same filter papers on which they were sprayed or were transferred to a clean surface, and, if they were transferred, after how long a period of exposure.   This information is necessary for a correct interpretation of the effect of temperature.

Whitcomb (1935) writes " the effectiveness of some materials varied directly with increase in temperature and others inversely.   Out of 34 materials or types of materials so far observed 16 were consistently more effective at 60 than at 80°F." The fact that the details about the technique used are not mentioned renders it impossible to weigh the importance of such evidence.

Potter and Gillham (1946) reviewed the work of Böttcher (1938, 1939) who found that both pyrethrum and rotenone were more toxic to honey bees as contact poisons at 20°C. than at 34·5°C. but the reverse was observed with nicotine.

Contact poison administered by dipping the organism in the liquid.

Craufurd-Benson (1938) while perfecting an immersion technique for testing liquid contact insecticides recognised that temperature " is the most important factor involved ; it is divisible into three parts (*a*) temperature before experiment, (*b*) temperature during experiment, i.e. the temperature of the insecticide at the time of application, and (*c*) temperature after experiment."   He was working with *Ahasverus advena*, Waltl (Col. Cucujidae) as test insect and a derris product as insecticide.   As regards the separate effects of the three components of temperature, five

immersion temperatures were tested (10°, 15°, 20°, 25°, and 30°C.) and it was consistently found that toxicity regularly increased with the increase in immersion temperature. Thus the effect of immersion temperature (*i.e.* temperature during treatment) in these experiments was the same as that of temperature during spraying in the case of Fleming (1933), Harries & others (1945) and David (1946). The effects of the two other components (*a*) and (*c*) have been unfortunately confounded. The insects bred at 20°C. (76 per cent. R.H.) and kept under the same conditions after treatment showed higher susceptibility than those bred and kept after treatment at 25°C. (75 per cent. R.H.). As in both sets of experiments the conditions after treatment were kept the same as those before treatment, it is not possible to decide whether the difference in susceptibility reflects the effects of temperature before treatment or that of temperatures after treatment. The combined effects of two components, however, is in the expected direction and lends support to the view of an increase in insect resistance with rise of temperature.

Probably it will not be out of place to include under this heading the effect of temperature on toxicity of fish poisons which are administered by contaminating the water in which the fishes are kept.

Powers (1920) described the increase with the rise of temperature in the toxicities of chlorides of lithium and ammonium to fishes and compared the temperature toxicity curve with temperature metabolism curves of other workers. More recently Gersdorff (1943) reported geometric increase in relative toxicity of rotenone and phenol to gold fish (*Carassius auratus*) with arithmetic increase in temperature. Obviously in these studies the fishes were kept continuously in the poison-containing water. That implies that the duration of treatment overlapped that of reaction and the temperature effect observed must be the resultant of the effect of temperature during treatment and that of temperature during reaction (after treatment). In view of these considerations these studies with fish poisons are probably analogous to those experiments in which *T. castaneum* adults were continuously confined over DDT films. Hence similar temperature effects are instructive.

In addition Hill (1909) measured the increase due to rise in temperature, in the action of nicotine on frog muscles by keeping two longitudinal halves of the same muscle in nicotine solutions at two temperatures. The contraction of the muscle was measured as a criterion for nicotine action. These cases support the general proposition of an increased toxic action due to an increase in temperature *during* treatment.

Contact poison administered by keeping the insect confined on poison film.

Lindquist & others (1945, 1946) have reported effects of temperature on knock-down and kill of houseflies, mosquitos and bed bugs exposed to DDT. The results of a few tests with pyrethrum and housefly are also reported. The technique consisted essentially in exposing the insects in screen-wire cages, glass jars or small wooden boxes which had been previously " treated with " DDT solution in kerosene, and recording the time required to effect complete knock-down. " When they were all down, the flies were divided and placed in clean untreated cages and held at various constant temperatures for observations on recovery and mortality". " In some cases they were exposed in treated cages for only short periods (not long enough to cause knock-down) before being transferred to the constant-temperature chambers". The main observations which at first sight appear to conflict with each other, can be summarised as follows :—

(1) When exposed continuously to pyrethrum or DDT-treated surfaces the knock-down with rise of temperature was slower in the case of houseflies exposed to DDT, and faster in the case of houseflies exposed to pyrethrum, and mosquitos and bed bugs exposed to DDT.

(2) In the case of houseflies " very little difference in mortality " is reported between flies exposed to DDT-treated surfaces at two different temperatures but in other cases a higher exposure temperature increases the mortality.

(3) The recovery is higher at higher temperatures except in the case of houseflies exposed to pyrethrum, where it is lower.

No attempt was made by Lindquist and his colleagues to reconcile these apparently conflicting results, but in view of the conclusions arrived at during the present investigations the discrepancies are more apparent than real, except for the observation of a lower mortality at lower temperatures in the case of houseflies exposed to pyrethrum. The slower knock-down at higher temperatures in the case of houseflies continuously exposed to DDT only indicates that in this case the increase in physiological resistance with temperature is probably the stronger factor and masks that of a greater pick-up at higher temperatures. This fact is also confirmed by the " very little " effect of exposure temperature. (Laug, 1946, also noted that " lowered temperature made the flies much more sensitive to DDT " film.) The lower mortality and greater recovery at lower temperatures in houseflies exposed until knock-down in pyrethrum-treated cages is inexplicable, and it is all the more surprising in view of the observation of Harries & others (1945) who, in all their experiments, found a higher kill at lower temperature in leafhoppers treated with various forms of pyrethrum, including residues. Eagleson (1942) who compared five temperatures also found higher recovery at higher temperatures in houseflies treated with pyrethrum or lethane spray.

Independent evidence in support of the suggestion that increased activity increases pick-up from DDT films has been provided by the work of Busvine and Barnes (1947) who report that the percentage mortality among bugs kept in motion for a specified period over a given deposit of DDT is greater than among stationary bugs exposed to the same deposit for the same period.

Sweetman (1945) published a paper with the title " Influence of Moisture and Temperature on residual kill of DDT ", but in reality the study is of the effects of environment on the rate of deterioration of DDT measured by biological assay methods. DDT films were stored for long periods under various conditions of temperature and moisture and then tested for their toxicity. This contribution, therefore, is not relevant to the present discussion, but it is certainly important in showing the effect of temperature, not upon the insects, but on the insecticide before treatment.

Contact poison administered as dust.

Potter and Gillham (1946) have reviewed the work of Gösswald (1933) who " investigated the action of pyrethrum dust on a number of forest pests under different conditions of temperature and humidity. He concluded that any given species was most resistant at its specific ' vital optimum ' temperature." Other references are to the work of Thalenhorst (1937), of Yarwood (1943) and of Parkin (1944). These three reported an increase in toxicity with rise of temperature, but the effects are combined with those due to temperature during treatment and those during reaction.

Poison administered with food (stomach poison).

Ellisor and Blair (1940) compared toxicities at 60° and 80°F. of four stomach poisons (synthetic cryolite, acid lead arsenate, basic copper arsenate, and calcium arsenate) to the velvet bean caterpillar (*Anticarsia gemmatalis*) and Southern army worm (*Prodenia eridania*). In all cases, except one (synthetic cryolite against *A. gemmatalis*), the toxicity was found to be greater at 60° than at 80°F. Böttcher (1938 & 1939) is also quoted by Potter & Gillham (1946) as having reported that pyrethrum extract tested as a stomach poison against the honey bee had a higher

toxic effect at 20°C. than at 34·5°C. These observations are not unexpected because in stomach poison experiments the temperature effect can take place only during the reaction of the poison. The effect of increased feeding activity during treatment might not exhibit itself if measured doses of poison are fed to each individual.

Poison administered as a fumigant.

There are a number of contributions on the effect of temperature on the toxicity of fumigants, the majority report higher toxicity at higher temperatures (Bertrand & others, 1919 ; Strand, 1927 ; Cotton, 1932 ; Jones, 1933 ; Lindgren, 1935 ; Gilyarov, 1942 ; Glass, 1944). Our knowledge of this subject, however, remains the same as it was when summarised by Shepard (1939). He writes :—

" Temperature is highly important in determining the effectiveness of a fumigant. Volatility increases whereas surface adsorption decreases with rising temperatures. The physiological activity of the insect is affected by temperature changes and through this fact susceptibility to fumigant action is modified. Bertrand & others (1919) found the action of chloropicrin on grain weevils to be accelerated by a small rise in temperature.

" Except when other factors interfere, fumigants are more toxic again at temperatures below about 10°C. Peters and Ganter (1935) pointed out that at 0°C. lower concentrations of hydrocyanic acid are required to kill the granary weevil (*Sitophilus granarius*, L.) than at 17°, giving as the reason that the physiological condition of the insects is very different at the two temperatures. Shepard & others (1937) showed that at temperatures below 10°C., the median lethal dose of a fumigant is reduced rapidly, probably because of the combined toxic effects of the fumigant and of the low temperature. Although a temperature of 0°C. by itself produces 50% mortality of flour beetles (*Tribolium confusum*) only after some days, apparently it exerts sufficient lethal action in five hours so that only as much chloropicrin need be used to give 50% kill at zero as at 25°. . . It is likely that the increased adsorption and reduced volatility of fumigants at low temperatures is sufficient in most cases in practice to offset the increased toxicity. Moore (1936) showed, however, that the mortality of an insect may sometimes be greater at the lower temperatures which favour physical gas adsorption by the insect rather than the higher ones which favour chemical and physiological action ".
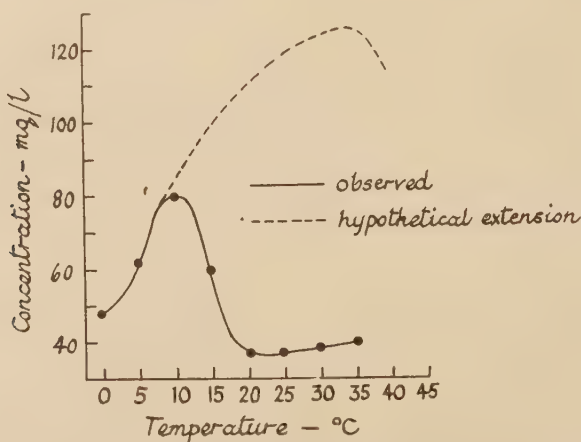
Fig. 11.—Effect of temperature upon median lethal concentration of ethylene dichloride for *Tribolium confusum*. (Data from Shepard & others, 1937.)

Despite the large amount and the nature of the data so far accumulated they do not warrant a discussion on the comparative merits of various possible explanations including those of the above passage. It can be shown that the observations cited in the above quotation, however, can also be explained with the help of the views being put forward in this paper.

The observations of Shepard & others (1937) on the toxicity of ethylene dichloride to *Tribolium confusum* are represented by the solid line in fig. 11. The interpretation of this curve given by Shepard, is that the toxicity of this compound usually decreases with the decrease in temperature up to about 10°C. but below that temperature the toxicity increases rapidly " probably because of the combined effects of the fumigant and of the low temperatures". The same curves, however, can be explained tentatively as follows :—starting from the lower temperature the first ascending portion of the curve indicates the increase in the insects' resistance with the rise in temperature. The upward curve would probably have continued approximately along the broken line but from about 10°C. onward the increase in temperature means also an increase in insect activity (especially respiration) resulting in increased doses of fumigant taken in by the insect. This increased dosage not only masked the upward trend of the insects' resistance but actually brought the graph down, apparently indicating increased susceptibility with rise in temperature. Further experimental work is needed to clear up this point.

Increased respiration as a result of increased temperature (or any other cause) resulting in an increased in-take of a fumigant hardly needs elaboration, nevertheless the literature is not free from confusion. Cotton (1932) while working on the relation of respiratory metabolism of insects to their susceptibility to fumigants came to the conclusion, " Any factor that increases the rate of metabolism increases the susceptibility of the insect to the action of a fumigant and vice versa. Of the known factors that increase the susceptibility of insects to fumigants the three most important are : an increase in temperature, an increase in the carbon dioxide content of the fumigation chamber ; and a decrease in the oxygen content of the fumigation chamber. The most effective results are obtained through a combination of these factors." This statement is very valuable from a practical point of view. But as a general conclusion applicable to all cases, no matter what the mode of administration, it is difficult to reconcile it with those conclusions arrived at with the film technique. All the evidence from contact poison experiments appears to favour the conclusion that increased metabolism due to increased temperature within the sub-lethal range, increases the basic resistance of the insect, the apparent increase in susceptibility observed under certain circumstances being most probably due to the insect receiving a higher dose of the insecticide. It is difficult to imagine that the effect of temperature on the reaction between insect and insecticide should be different if the insecticide enters the system as vapour through tracheal walls instead of passing through the outer body wall as a contact poison.

This foregoing survey of the literature on the effect of temperature on the toxicity of various poisons administered in various ways promises the possibility of co-ordinating isolated and apparently unrelated observations into a few basic generalisations. As toxicity is the result of interaction between two fundamentally different groups of materials, *i.e.*, between various species of living organisms on one side and various types of chemicals on the other, the conceptions developed and discussed above represent only the aspect applicable to one group, *i.e.*, that of the living organism. The effect of temperature on toxicity through physical or chemical changes in the poison itself is an entirely separate aspect which, but for a reference to the paper by Sweetman (1945), has not been touched in the foregoing pages. This latter aspect must be kept in view in more critical analyses of temperature effects. Although the range of temperature within which toxicity is tested is generally too narrow for chemical change, temperature is likely to affect toxicity through such physical changes in the poison as its volatility, crystal size, and other characteristics.

Summary.

1. A series of exploratory experiments on the relationship between temperature and toxicity of DDT films to adults of *Tribolium castaneum*, and larvae of *Plutella maculipennis*, are described. The main conclusions with *T. castaneum* are :—

(*a*) When the insects are continuously kept on the film at different temperatures there is a higher kill at higher temperatures.

(*b*) When the insects are exposed to the film for about 24 hours at the same temperature and then kept away from it at different temperatures there is a higher kill at the lower temperature.

(*c*) When the insects are kept on the film at different temperatures for about 24 hours and then kept away from the film for reaction at the same temperature, there is a higher kill in those kept on the film at the higher temperature.

(*a*) and (*b*) above apply equally to larvae of *P. maculipennis* but (*c*) is reversed. The probable cause of this reversal appears to be the observed fact that at higher temperatures these larvae cover the film with much more silk thread and thus avoid contact to a greater extent than at lower temperatures.

2. A review of literature, in the light of the conclusions arrived at, indicate that many of the observations made upon the temperature-toxicity relationship can be accounted for by the following generalisations :—

(*a*) Insect resistance to poisons changes with temperature as do its other vital activities, increasing up to a critical degree and afterwards declining.

(*b*) The amount of poison reaching the site of action in unit time also varies with the temperature, generally *but not always*, increasing with its rise. Insect activity, especially locomotor and respiratory, may play an important part in these effects.

(*c*) The apparent effects of temperature on insecticidal action is the combination of these two factors, namely, resistance and pick-up.

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G
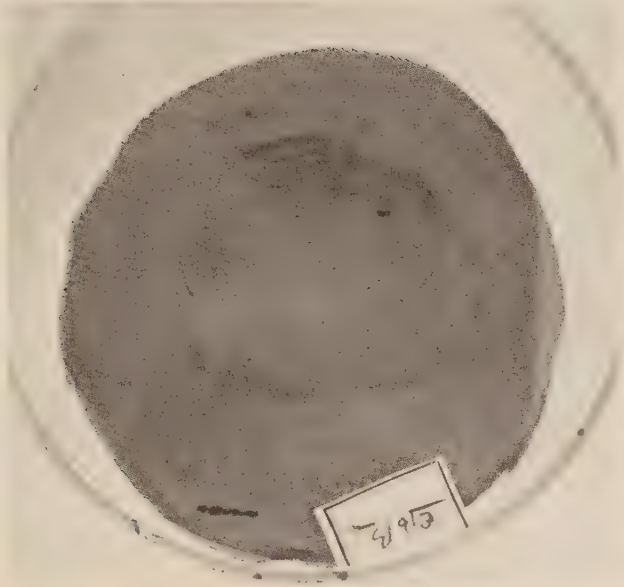
(*a*) Photograph of a circle of bolting silk over which
Plutella maculipennis larvae were kept confined at
80° F. (Note the patch in the middle which shows
the silk secreted by the larvae.)
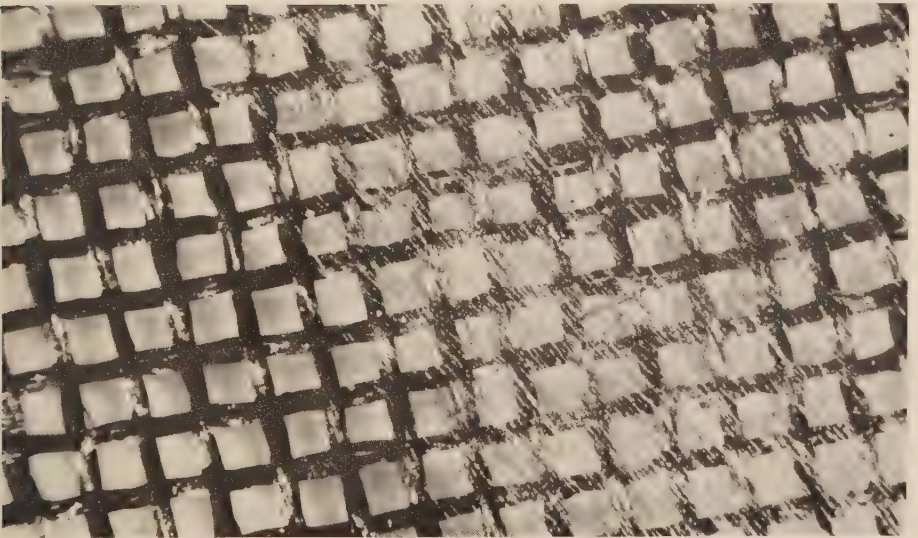
(*b*) Photomicrograph of a portion of (*a*). The silk secreted by the larvae covers the
bolting silk. (Photograph taken by means of reflected light.)
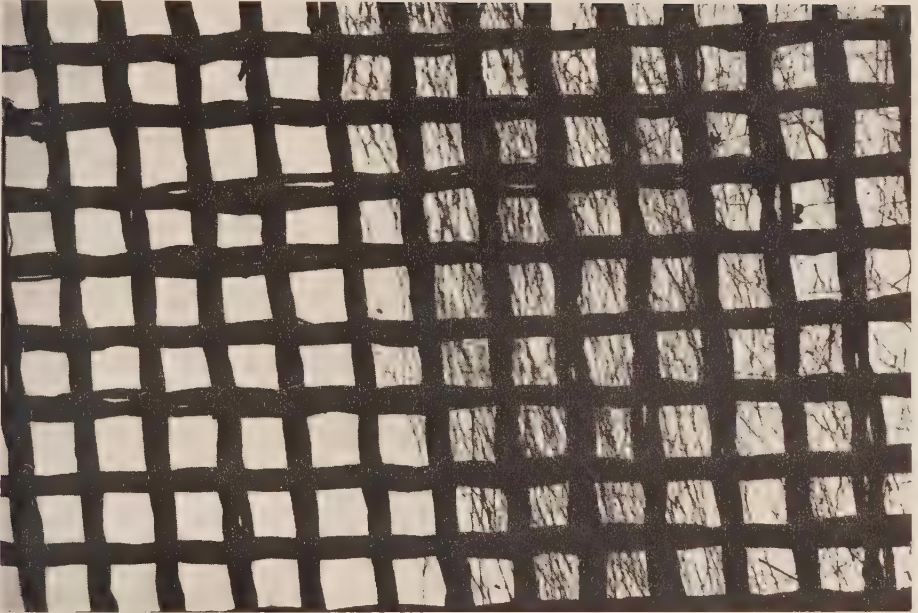
(a) The same as in Plate III (b) photographed by transmitted light.
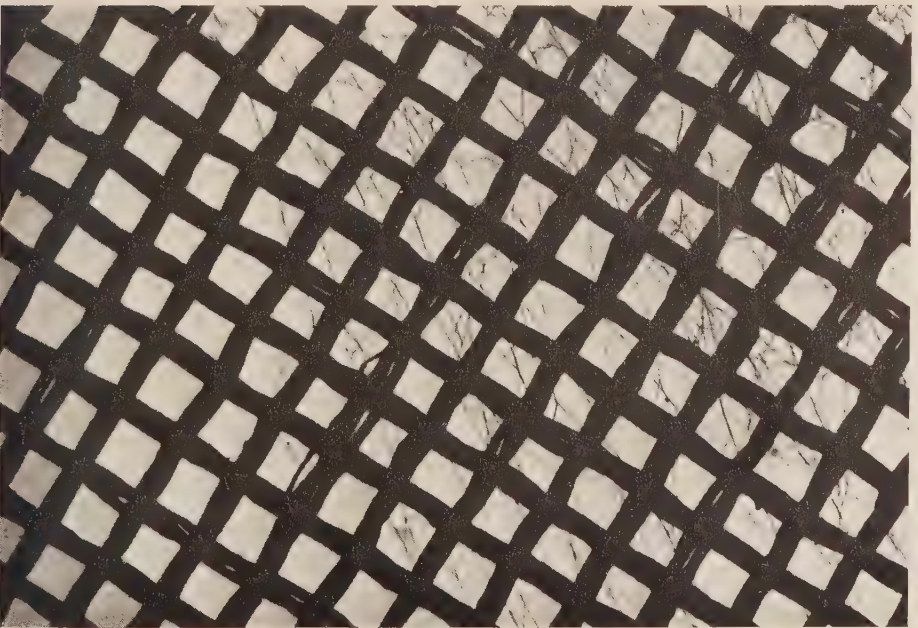
(b) Shows the amount of silk secreted when larvae of *Plutella maculipennis* were kept at lower temperature in the basement room.

STUDIES ON WHEAT BULB FLY, *LEPTOHYLEMYIA COARCTATA*, FALL.

III.—A SURVEY OF INFESTATION IN YORKSHIRE.

By H. C. GOUGH, Ph.D.

National Agricultural Advisory Service.

The author (1946, 1947a) has described the biology of the Wheat Bulb Fly, *Leptohylemyia coarctata*, Fall., in Yorkshire and given detailed estimates of its numbers in certain specified areas where it occurred as a pest. It was considered desirable to supplement this information by a more extensive survey in order

(i) To assess the extent and seriousness of the problem.

(ii) To discover if there were areas other than those already known where wheat bulb fly was a pest.

(iii) To attempt to ascertain the characteristics of the areas where the fly was a pest and to what extent the fly occurred where it was not a pest.

(iv) To obtain a standard with which future information might be compared if at some later date this was considered necessary.

General Plan of the Survey.

Attacked plants cannot be identified with certainty before the beginning of March, so that it would not be possible to commence the survey earlier. By the beginning of April most wheat crops are so thick that it is difficult to find and count all damaged shoots. The larvae have destroyed and left one shoot by this date, and, in addition, wireworm damage is becoming more evident so that the total number of damaged shoots per unit area becomes increasingly greater in relation to the number of shoots containing larvae. As only a limited number of shoots could be examined in the time available, accuracy decreased rapidly about the end of March. It was therefore decided to limit the period to this month only and naturally this affected the scope of the survey.

The method of selection of fields presented some difficulty. A preliminary survey was made in 1944 in which fields were examined at approximately 10-mile intervals on main roads throughout the county. This method suffered from the disadvantage that either it was not possible to obtain reliable information about the previous crop, date of sowing, etc., or, a very considerable amount of time was lost in finding the owner of the field to obtain such information.

For the main survey in 1945 it was therefore decided to base the selection on farms, and a total of 100 farms spaced as evenly as possible over the arable areas of the county was aimed at. These included some areas which had been predominantly grass before the war and only the upland areas of the Pennines and North Yorkshire Moors were excluded. The farmers chosen were generally well known to the Leeds University Department of Agriculture or to the district agricultural officer. Although this selection was not strictly at random as is desirable in such a survey, it was not likely to influence the actual numbers of Wheat Bulb Fly recorded and it did have obvious advantages.

As far as possible two fields on each farm were examined. Wheat after permanent grass was excluded owing to the probability of wireworm damage. If a farmer had more than two fields of wheat, two were chosen to include a variety of previous crops. For example if a farmer had three wheat fields after potatoes and one after seeds, one of each was examined even if one rotation was more characteristic of the district than the other. This selection might perhaps have introduced some bias into the

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results but in fact the number of occasions when there was a choice was few and there were so many other sources of error that there was little point in compensating or allowing for any one which could be measured. Another possible source of error was the inclusion of rye instead of wheat on certain farms where little wheat was grown. Although the total quantity of rye grown in the county in relation to wheat is very small, the number of rye fields examined was 10 (out of 136) and 5 of these occurred in districts where Wheat Bulb Fly was a pest. In the general discussion and tables all these fields are referred to as wheat.

The 100 farms chosen represented about one farm to 30 square miles of the arable areas of the county, but it was only possible to visit 86 farms altogether including 136 fields. The total acreage of these fields was nearly 1,300, representing about 0·6 per cent. of the total Yorkshire wheat acreage for that year.

Technique.

Within the field 10 2-ft. squares were examined at random. Squares were used to permit a direct comparison between broadcast and drilled crops. All damaged central shoots were counted and a maximum of 10 removed from each square. An additional 10 plants were taken at random near each square, but outside it. All these (*i.e.*, 100 random plants and up to 100 damaged shoots) were brought back to the laboratory for examination. The number of Wheat Bulb Fly (or other) larvae was recorded and if the total number of damaged shoots counted in the field was greater than the number brought back, the total numbers of Wheat Bulb Fly larvae were estimated. For example, a series of damaged shoots in the field might be :— 7, 9, 3, 15, 22, 11, 5, 0, 5, 8, making a total of 85. Taking the first 10 from each square means that 67 shoots would be brought back. If 42 out of these 67 contained Wheat Bulb Fly larvae, the total number of larvae in the 10 squares was assumed to be the fraction $\dfrac{42 \times 85}{67} = 53$. This is an average of 5·3 per frame or about 57,000 larvae per acre. The 100 plants taken at random gave an approximate idea of the percentage of plants attacked. As far as possible the cause of damage to the shoots not containing Wheat Bulb Fly larvae was ascertained. A number had usually been damaged by Wheat Bulb Fly larvae which had by then moved to another shoot, some contained other insect larvae (Gough, 1947b) and others had been attacked by wireworm.

The method of estimation is admittedly approximate but it was considered to be accurate enough to place a field in a certain category, and increasing the number of samples or the number of plants examined would necessarily have reduced the number of fields visited in the limited time available. The possibility of increasing the time for field work by storing material in a refrigerator was investigated in 1946, and it was found quite practicable to keep wheat containing larvae satisfactorily for at least two weeks in this way.

Results.

(i) *Analysis.*

In order to select the most suitable limits for the different categories, the distribution of fields for each increment of 10,000 larvae per acre was examined. The result of this analysis is shown in fig. 1, together with the percentage of plants attacked, plotted against the number of larvae per acre.

It can be seen that there are many fields with less than 10,000 larvae per acre. There are many fewer in the next group, 11,000–20,000/acre, and the numbers then gradually decrease to about the 100,000/acre mark, above which there are only isolated fields. Moreover, about the level of 100,000/acre the percentage of plants

attacked appears to rise to about 35–45, and does not increase above this figure. 10,000 and 100,000 larvae per acre therefore suggested themselves as convenient limits, and three main categories were selected as follows :—

Under 10,000 larvae per acre ... Low ; damage would not be noticed in the field at this level.

11,000–100,000 larvae per acre Moderate ; damage is unlikely to be noticed except in an extremely poor crop.

Over 100,000 larvae per acre ... High ; although at its lower levels, economic damage is unlikely under normal circumstances, it is an indication that the local conditions are favourable for Wheat Bulb Fly and damage is likely in the district.
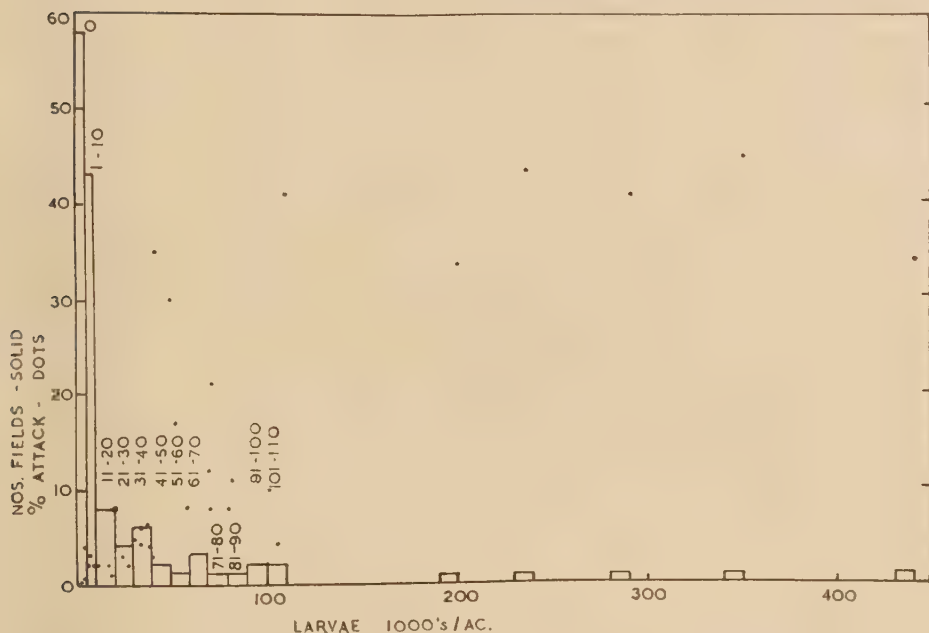
Fig. 1.—Distribution of fields at different levels of infestation and percentage plants attacked in relation to numbers of larvae per acre.

Most of the results have been analysed in these three categories, but for certain purposes two other categories are introduced :—

(a) No larvae found. Although the absence of larvae from a few square yards sampled in one or two fields representing a very large district is a quite inadequate basis on which to decide that it is entirely absent from that district, this category did help in distinguishing between certain areas where the fly was present in varying degrees but never, or rarely, as a pest.

(b) Over 300,000 larvae per acre. Gough (1947a) has suggested this figure tentatively as a critical one below which failures or serious losses are unlikely to occur except under the most unfavourable circumstances when a poor crop would be expected even in the absence of the fly, and above which failure or serious losses were quite probable except under favourable circumstances.

(ii) *Distribution.*

All the information derived from the 1945 survey, together with 75 records from the 1944 survey, records of failures and occurrences reported to the advisory entomologist during the period 1935–46, and fields in special areas studied by Gough (1946, 1947a), was plotted according to the degree of infestation on a map of the county on a scale ¼ in. to the mile. A copy of the map is reproduced in fig. 2. Most areas and places mentioned in the text are indicated on the map. The field technique adopted in 1944 was similar to that described for 1945 except that the area examined was not a precise 2 ft. square but included a semi-circle limited by the reach of the arms of the observer standing in one spot—about 6 sq. ft. Only a small proportion of damaged plants was brought back to check the identity of the cause of damage. The field was then placed in one of 4 categories roughly corresponding to the three used in the 1945 survey and the group " no larvae found ". The survey was made in April so that for reasons already given it could not have been very accurate.

Fig. 2.—Distribution of Wheat Bulb Fly in Yorkshire.

No attempt has been made to delimit the different areas precisely on the map but a list of parishes is given below for each main area including all those where there have been records of a high count in the survey or a crop failure. The total acreages given in Table I are derived by adding up the acreages of those parishes where at least two failures or high counts have been recorded. The total will naturally include

areas where conditions are not similar to the surrounding district and where Wheat Bulb Fly is not a pest, but these will be balanced to some extent by surrounding areas where the records and failures are so few that they have been omitted from the list. The possible value of these figures is discussed in a later part of this paper.

1. *Selby Area.*

The soil is light, ranging from blowing sand to a light loam. Damage occurs almost exclusively after potatoes. This area has been intensively investigated and the limits are fairly well known. The following parishes are involved :—

West Riding. Ryther cum Ozendyke, Cawood, Wistow, Selby, Hambleton, Thorpe Willoughby, Brayton, Gateforth, Barlow, Camblesforth, Rawcliffe Bridge.

East Riding. Naburn, Stillingfleet, Deighton, Thorganby, Barlby, Cliffe (including South Duffield), North Duffield, Hemingborough, Barmby, Asselby, Ellerton with Aughton, Bubwith.

2. *Pontefract Area.*

Soil varying from blowing sand at Balne to heavy clay at West Hardwick. Damage has occurred after fallows on heavy soil, potatoes on light soil, and peas. The writer has not had the opportunity of seeing failures after peas but they have been recorded commonly by Mr. H. W. Thompson, who states that attacks mainly occur after early peas for pulling green. As the haulms are pulled up during picking, the ground is left more or less exposed even if it is not ploughed immediately. In other parts of the county where peas are grown on a field scale they are usually harvested later for drying. The following parishes (all in the West Riding) are involved :—

Normanton, Featherstone with West Hardwick, Womersley, Balne.

3. *Warp Area.*

The soil is medium to the south of the Humber, where damage occurs after potatoes, and heavy to the north of the Humber, where damage occurs after fallows. Failures or high counts have only been recorded on one or two adjacent farms in each of the two parishes involved. Neither of these parishes was included in the survey :—

West Riding. Goole Fields.

East Riding. Blacktoft.

4. *Holderness.*

Very heavy soil where damage only occurs after fallows. This area has not been well surveyed and the acreage estimate is uncertain. The parishes involved (all in the East Riding) are :—

Sunk Island, Thorngumbald, Easington.

5. *Cleveland.*

Again a very variable soil. In the north-eastern part of the valley damage occurs mainly after fallows on heavy land, but in the south-western part there are some cases of damage after potatoes on light land. This area also has not been well surveyed and the acreage estimate is only approximate. The parishes involved (all in the North Riding) are :—

Redcar, Eston, Stokesley, Maltby, West Harlsey, Catterick.

6. *Dirtness Bridge.*

Soil of black sand on the Lincolnshire border. Damage occurs after potatoes and though the records only relate to one farm, it seems probable that similar damage occurs on neighbouring farms.

H. C. GOUGH.

Additional information about these areas is tabulated in Table I.   In this and all other Tables, the number of fields and mean larval counts are derived from the 1945 survey only.

TABLE I.

District	Estimated acreage	Mean larval count (1,000's acre) after "susceptible" crops	Mean larval count (1,000's acre) after other crops	No. of failures reported 1935–1945
		(Total No. : fields in brackets)		
Selby	50,000	143 (7)	16 (7)	35
Pontefract ...	10,000	55 (4)	12 (4)	6
Warp	5,000	— —	— —	3
Holderness ...	20,000	228 (4)	1 (2)	2
Cleveland	1,000	65 (5)	1 (9)	6
Dirtness Bridge ...	1,000	— —	— —	2
Total	106,000	— —	— —	54

The mean larval counts for Cleveland are based on a larger area than that indicated in the column headed " estimated acreage ". Owing to lack of detailed knowledge of the distribution of the fly here, all counts between " islands " of known infested areas have been included. Attention must be drawn to the fact that the relative numbers of fields after " susceptible " (potatoes, peas, fallow) and other crops vary for different areas and that the previous crop exercised some influence on the selection of fields for the survey. Because of this, direct comparisons of mean larval counts between areas on wheat after all crops are not possible. For example, in the Cleveland area, the mean larval count for all wheat fields examined would only be about 24,000/acre, though even this is considerably higher than any of the means for the areas where Wheat Bulb Fly does not occur as a pest (Table II). The figures in both tables are given mainly to show general indications and to provide some evidence that the limits of the Wheat Bulb Fly areas described here have a factual basis.

One of the objects of the survey was to determine to what extent Wheat Bulb Fly occurred in the areas where it was not a pest. Did it, for example, occur in moderate numbers everywhere, or was it entirely absent, or something intermediate between these two extremes ?

The preliminary survey in 1944 was confined mainly to the areas where Wheat Bulb Fly was not known to occur, and out of 58 fields in such areas, 2 were placed in a high category, 7 in a medium category, 9 in a low category, and in 40 fields no larvae were found at all.

The results for such areas for the 1945 survey are set out in Table II. For the plain of York there were sufficient fields after " susceptible " crops (potatoes, peas, fallow) that it was possible to analyse these separately. In other areas there were only a few fields in this category.

It can be seen from Table II that the Yorkshire Wolds is the only arable district where Wheat Bulb Fly is virtually absent. This is doubtless due in part to the fairly rigid rotation practised there in which wheat almost invariably follows late ploughed seeds or a cereal. There appears to be a tendency for moderate numbers to develop

TABLE II.

Numbers of fields in areas where Wheat Bulb Fly does not occur as a pest, classified according to larval count.

Area	Number of Fields					Mean population 1,000's/acre
	No larvae found	1–10,000 /acre	11–100,000 /acre	Over 100,000 /acre	Total	
Plain of York excluding Selby area; after "susceptible" crops	2	8	5	0	15	7·4
Plain of York excluding Selby area; after other crops	14	8	0	0	22	1·1
Southern Industrial area (Coal measures)	8	8	4	0	20	4·3
Yorkshire Wolds	16	2	0	0	18	0·3
All other areas	9	4	1	0	14	1·4
Totals	49	30	10	0	89	—

after susceptible crops in the northern part of the plain of York and there are isolated cases of failure in this area.

(iii) *Influence of previous Crop.*

In Table III the fields are grouped and classified according to the previous crop. In general this table follows the expected lines and does not reveal any unusual trends. It is interesting to note that the fly is present in about one-third of all wheat after cereal fields examined. The mean population of all these fields is about 2,000 larvae/acre and although this is a very low figure, it does suggest that even if susceptible rotations were avoided for a few years, as was proposed by one district officer, there would probably always be a nucleus capable of building up the original population.

The wheat after clover and seeds section, is rather more heterogeneous than would appear at first sight. The important consideration is the date of breaking up and it was rarely possible to ascertain this precisely. The one field in the highest category which had 105,000 larvae/acre had been ploughed in the third week of July. Only for two of the five fields in the 11,000–100,000/acre category was the approximate date of ploughing known. In one case it was July (100,000/acre), and in the other case August (44,000/acre). These dates are neither sufficiently numerous nor accurate enough to assist in narrowing down the date of oviposition.

For wheat after potatoes nearly half the fields have counts in the two high categories. Only four of the potato fields were in parishes where Wheat Bulb Fly failures had been reported previously, but most of the moderate and high counts occurred in districts where they might have been expected from the rotation and type of soil. The majority (18 out of 28 fields) were on light soil, but the numbers are not sufficient for useful comparisons between different types of soil.

Seven out of the eight wheat fields after fallow were in the two higher categories. This uniformity is to be expected as only exceptionally heavy soils are fallowed as a regular practice. One interesting field in this group was in a district in which practically

no land had been under the plough since the 1914–18 War. The count was only 24,000/acre, but, again, it does rather indicate the possibility of the fly always being present in sufficient numbers to build up where conditions are favourable.

TABLE III.

Influence of previous crop on Wheat Bulb Fly populations.

| Previous crop | No. of Fields with | | | | | Mean population (1,000's/acre) |
| | 0 | 1–10 | 11–100 | Over 100 | Total | |
	Thousands per acre					
Cereals and flax ...	23	11	1	0	35	2
Clover and seeds ...	16	13	5	1	35	12
Potatoes	6	12	11	3	32	40
Fallow	1	0	4	3	8	126
Peas	5	3	5	0	13	15
Beans	4	0	0	0	4	—
Miscellaneous, roots, turnip, rape, etc. ...	4	2	1	0	7	4
Totals	59	41	27	7	134	22

Discussion.

In spite of the enormous possible errors and the relative paucity of information, it was considered important to try to estimate the total wheat acreage in Yorkshire which is liable to attack by Wheat Bulb Fly. In order to make full use of the many surveys of pests and diseases of agricultural crops at present contemplated or in operation, some estimate of the damage concerned, however approximate, will assist in deciding the priority to be given to research on the problem concerned. This aspect is therefore discussed at some length, as it is felt that the problems and difficulties encountered may be of value in helping other workers to plan surveys so that some of the difficulties may be minimised.

In an earlier section the figure of 106,000 acres of land was suggested as an approximate estimate of the districts in Yorkshire where Wheat Bulb Fly attack was likely to be serious. All of these are arable areas with only small proportions of permanent grass, and uncultivated land. The fraction of land in wheat, in any one year, in these areas is assumed to be about one-seventh or about 15,000 acres. Of this, perhaps one-half to two-thirds will be after a susceptible crop and a figure of 7,000–10,000 acres is therefore reached, which represents the amount of wheat, and rye, in any one year in areas where Wheat Bulb Fly attack is likely to be serious enough to cause some failures and probably a general loss in yield in an unfavourable season. This figure might be regarded as an upper limit, as some fields usually yield satisfactorily in these districts, and on the better land especially, wheat has remarkable powers of recovery. The absence of yields for different levels of infestation makes it impossible to transform these vague acreage figures into terms of wheat tonnage lost. The possibility of trying to obtain yields for the survey fields was considered, but as there were so few fields in the important higher categories, and the normal yields would vary from place to place, the idea was rejected. The lack of exact knowledge of the ultimate effect of the pest on yield is one of the weakest points of this survey and the writer commends the consideration of this aspect to workers engaged on similar problems.

Another estimate was made by calculating the percentage of wheat fields in the higher categories and then transforming it to an acreage basis. The details are given in Table IV.

In the county as a whole 8 out of 136 fields have numbers high enough to be considered serious. This is about 6 per cent. and represents, therefore, approximately 12,000 acres. There are only two fields in the critical over-300,000/acre group which represent about 1·5 per cent. or approximately 3,000 acres.

The possible errors in this calculation are, of course, enormous, but the first figure suggested does seem to be in reasonable agreement with the upper limit of 7–10,000/acre already mentioned, and for want of more accurate information it seems reasonable to assume that in an unfavourable season some crop loss, amounting at least to a few cwt./acre is probable on, say, two or three thousand acres of wheat in Yorkshire. Although this is perhaps relatively small when compared with the total amount of wheat produced in the county, the fact that the damage is confined to certain districts makes it a very serious problem in those districts.

TABLE IV.

Riding	No. of farms visited	No. of fields examined	Total acreage examined	Total wheat acreage (1945)	No. of fields with populations of			
					0–10	11–100	101–300	over 300
					larvae—1,000's per acre			
West	40	70	632	72,000	50	18	1	1
East	29	46	478	77,000	36	6	3	1
North	17	20	174	45,000	15	3	2	0
Totals	86	136	1,284	194,000	101	27	6	2

The area of wheat which is a more or less complete failure is even more difficult to determine. The advisory records of the province for 1935–1946 in Table V show the following approximate acreage of failures reported to or seen by the advisory entomologist.

TABLE V.

Year	No. of fields	Acreage	Year	No. of fields	Acreage
1935	3	20	1941	11	80
1936	5	30	1942	2	15
1937	0	0	1943	11	80
1938	3	20	1944	26	200
1939	0	0	1945	10	50
1940	2	20	1946	1	5

The proportion of failures reported, compared with the actual acreage which failed, will vary somewhat. It is probably similar for each of the years up to 1943 when the work on the problem was commenced and when district officers were specially asked to look out for the pest. There is no doubt, however, that there was a real and large increase in 1944 even over the previous year, when attacks were relatively serious. Probably the total acreage which failed in that and subsequent years was at least twice the acreage reported and a rather higher proportion in other years.

The point raised on page 267 under (i) has been covered above and that in the second part of (iii) on pages 272–273.

As regards the other points :—

(ii). No counts were recorded during the survey over the 100,000/acre limit which were not in or on the outskirts of areas already known to be liable to failures.   One farm at Dunnington about midway down the East Riding coast had two fields of wheat after fallow with counts of 40,000 and 50,000 larvae per acre on a very poor crop, and as fallow-wheat was apparently common in the district, a more intensive search might have revealed higher counts in other fields.   Apart from this possibility, it seems almost certain that all the larger areas where Wheat Bulb Fly is likely to occur as a pest in Yorkshire are known.

(iii, first part).   The two general types of conditions which favour attack have already been described, both in this paper and by Gough (1946).   These conditions are (1) wheat following a fallow on heavy land, and (2) wheat following potatoes on light to medium land.   Even these circumstances do not invariably pre-dispose land to attack.   During the 1945 survey, five fields were examined in the Holme on Spalding Moor—Market Weighton area.   This is carrot land where wheat and rye commonly follow potatoes on blowing sand, and the general conditions, except that the soil is perhaps somewhat lighter, are very similar to those at South Duffield where Wheat Bulb Fly damage occurs every year.   Only one larva was found in all these fields, and this fact was considered sufficiently remarkable to examine further fields in 1946.   In March, 1946, 7 fields of wheat or rye after potatoes on 5 farms were inspected.   One field had no larvae and all the others were in the low category with a mean of 4,000 larvae/acre.   It does seem then, that the rotation of wheat or rye after potatoes on light land is not in itself sufficient to build up a population.

The two sets of circumstances which favour oviposition are so different that it is tempting to consider the possibility of two different biological races.   In support of this idea Gough (1947a) has already recorded that the numbers of eggs and larvae were low in wheat after potatoes at Sunk Island although wheat after fallow was heavily infested, but it had never been possible to examine fallow wheat in the Selby area.   In 1945, however, it was noted that certain fields at North Duffield, only about one mile from fields known to be infested at South Duffield, were being fallowed.   They had been ploughed out of permanent grass some years previously and had carried a succession of cereal crops with the result that weeds became a serious problem, and it was necessary to fallow.   The soil was heavy and it was thought that the conditions presented an interesting contrast to the severely infested area at South Duffield.

Three of these wheat after fallow fields were carefully examined in March, 1946, but not a single Wheat Bulb Fly larva was found.   There was a fair amount of permanent grass here before the 1939–1945 war, but one would have expected at least a small Wheat Bulb Fly population which might have increased to the extent of being noticeable under favourable circumstances.   The land was rather wetter than the surrounding area, and this may have been a more important factor than the heaviness of the soil.

In the Pontefract area, however, the two types of attack both occur within fairly small areas, a fact which does not support, although equally it does not disprove, the possibility of two races, and very much more evidence is required on the point.

Summary.

A quantitative survey of Wheat Bulb Fly (*Leptohylemyia coarctata*) was made in Yorkshire in 1945, and the distribution of the fly is discussed in relation to general observations and records in the years 1935–1946.   The distribution is shown in the map (fig. 2).

One hundred and thirty-six fields on 86 farms were examined and Wheat Bulb Fly was present in over half of the farms and fields ; in 8 fields (6 per cent.) it was

present in moderately high numbers, and in 2 fields (1·5 per cent.) it was present in numbers sufficiently high to be likely to cause a failure in a bad wheat year. It was estimated that two or three thousand acres of wheat and rye in Yorkshire are likely to have a reduced crop as a result of Wheat Bulb Fly attack in an unfavourable season. Over half the dangerous area is near Selby. The only large area where Wheat Bulb Fly was virtually absent was the Yorkshire wolds.

High larval counts and wheat failures occur mainly on light land after potatoes and heavy land after fallows, but at least one area was found where wheat and rye regularly followed potatoes on light land but where Wheat Bulb Fly was only present in very low numbers.

Acknowledgements.

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LABORATORY EXPERIMENTS ON THE EFFECT OF DDT AND BHC ON CERTAIN APHIDOPHAGOUS INSECTS AND THEIR HOSTS.

By M. J. WAY.

Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts.

The spectacular results obtained in the biological control of certain insect pests in California and Hawaii have been followed by indifferent results in later applications of this method of control to other pest insects. It is realised that biological methods as such are not generally applicable in effecting complete control, but abundant evidence is available to show that biological agencies are of everyday importance in reducing the size of pest insect populations. The relationships between Aphid populations and their parasites have been studied by Ripper (1944) (*Brevicoryne brassicae*, L.), Broadbent—unpublished (*Myzus persicae*, Sulz.) and Arthur (1944) (*Myzus kaltenbachi*, Schout.). The biology and feeding habits of Syrphid and Coccinellid predators are described by Campbell and Davidson (1924), Clausen (1916), Hawkes (1920), Heinze (1939) and Metcalf (1916). The evidence from these sources shows that entomophagous insects can cause an appreciable reduction in the case of Aphids and may under suitable conditions effect 100 per cent. control (Barnes, 1931) though cases where such insects are apparently of little importance are also recorded (Petherbridge & Mellor, 1936).

The use of insecticides for insect pest control imposes further complications on the already complex host-parasite relationship. The ideal insecticide is a selective poison which kills a high percentage of the pest whilst leaving its natural enemies unaffected (Ripper, 1944) but it is clear that most of the insecticides in use today do not possess such desirable properties. Insecticides such as DDT* and BHC† appear to be destructive to a wide range of insect species and their possible danger to beneficial insects is enhanced by the fact that they act as both stomach and contact poisons, by their properties of persistence and by the methods used for widespread application (Brooks, 1947).

Although destructive effects of spray treatments on parasites and predators have often been recorded (Driggers & Pepper, 1936, Driggers & O'Neill, 1938, Steiner, 1938, Cox, 1942), it is not always clear how far this has favoured the pest since the latter has also been reduced in numbers by the insecticide.

The danger of insecticides is most apparent in cases where they are more destructive to the natural enemies than to the pest or potential pest. This has been chiefly noticeable when DDT, used for the control of codling moth and other pests, has often caused a considerable increase in numbers of the red spider mite through destruction of its predators. Similar observations have been made on several other pest insects.

Steiner & others (1944) observed that treatment of apples with DDT for codling moth control resulted in a noticeable abundance of the " European " red mite, *Paratetranychus pilosus*, C. & F., in the following July, while at harvest time large populations of the common red spider (*Tetranychus* spp.) were found on fruit and bark of all DDT-sprayed trees but were difficult to find on all other trees. Weigel (1944), using a 2 per cent. aerosol, 3 per cent. dust and a spray (0·2 lb. DDT per 100 gals.) against vegetable pests, found that DDT failed against *Tetranychus* spp. on

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*Dichlorodiphenyl trichloroethane.

†Hexachlorocyclohexane (Benzene hexachloride)—the commercial product consisting of a mixture of isomers of which about 10 per cent. is active γ-isomer.

radish and bean and also against the citrus mealybug, *Pseudococcus citri*, Risso.   An increase in mites after DDT treatment has been confirmed by Swanson and Michelbacher (1945) (*Tetranychus bimaculatus*, Harvey), Taylor (1945), (*P. pilosus* and *Bryobia praetiosa*, Koch), Hough & others (1945) (*P. pilosus* and *Tetranychus schoenei*, McG.), and Smith (1945) (*Tetranychus atlanticus*, McG.).   The latter observed that *Aphis gossypii*, Glov., was unaffected by a DDT dust while the predators, *Geocoris pallens*, Stål (Lygaeidae) and *Nabis ferus*, L. (Reduviidae) had almost entirely disappeared within 24 hours of treatment.   Hough & others (1945) found that DDT spray (1·5–2 lbs. DDT per 100 gals.) had little or no effect against the adult females of the Comstock mealybug (*Pseudococcus comstocki*, Kuw.).   At 0·5 lb. per 100 gals., it was extremely toxic and quick acting against adults of the mealybug parasite—*Pseudaphycus* spp.   Although the extent of parasitism in the field was depressed by DDT treatments, it increased rapidly after these were discontinued in August.

Woodside (1946) considered that the use of DDT made mite control necessary and, in America, the Bureau of Pest Control of the California Fruit Growers Exchange (Anon. 1947) issued a warning that, while DDT has shown itself effective against citricola scale and citrus thrips, the effect on the Vedalia ladybird beetle has permitted cottony cushion scale to increase in certain groves in an alarming way.

Laboratory experiments carried out by Peterson (1947) showed DDT to be highly toxic to the adults of certain insects parasitic on the oriental fruit moth and strawberry leaf roller larvae.   A film of 0·01 per cent. DDT on foliage or glass caused 100 per cent. kill of *Cremastus cookii*, Weed, and *C. forbesi*, Weed (Ichneumonidae) and *Nemorilla floralis*, Fall. (Tachinidae).   *Macrocentrus ancylivorus*, Rohw. (Braconidae) and *Archytas apicifera*, Wlk. (Tachinidae) showed total mortality at a strength of 0·002 per cent. DDT.   Persistence tests showed that DDT on peach foliage remained toxic for 4–7 weeks.

Work in Britain has given general confirmation of these findings.   Wilson (1946) reported that a 0·1 per cent. DDT emulsion destroyed Coccinellids and adults of *Encarsia formosa*, Gah. (the greenhouse whitefly parasite) but had little effect on certain Aphid species and none on the greenhouse red spider (*Tetranychus telarius*, L.). Potter and Perkins (1946) observed that Coccinellids and species of *Aphidius* parasitic on Aphids were destroyed by 5 per cent. DDT dust and 0·2 per cent. DDT spray in the field, although Syrphid larvae were unaffected.   According to Massee (private communication), an apple orchard sprayed in March with a DDT emulsion at 2 lbs. per 100 gals. showed no living insects for three weeks, many beneficial species, notably Carabids, being destroyed.   An important observation was that, by blossom time, the beneficial insect population in this particular orchard compared favourably with that of nearby untreated orchards.   Massee also observed that *Coccinella septempunctata*, L., and *Adalia bipunctata*, L., were not seriously affected by 3 per cent. DDT-china clay dust and 0·1 per cent. DDT wettable powder spray, applied to apples at weekly intervals from early June.   Massee (1947) mentions that some 30 insects, capable of feeding on fruit tree red spider in its various stages, are susceptible to DDT; in a private communication he states that the most important of these is the black-kneed predatory Capsid (*Blepharidopterus angulatus*, Fall.).

Published data on the toxicity of BHC are confined to those of Taylor (1945) who observed an increase of mite populations of *P. pilosus* and *Bryobia praetiosa*, Koch, after treating apples with a spray containing 1 lb. BHC (5 per cent. γ-isomer) per 100 gals.   This increase was smaller than that caused by comparable DDT treatments.

The present work developed as a result of observations made during field experiments in 1945 (Potter & Perkins, 1946).   DDT sprayed and dusted on various brassica crops had no apparent effect on the mealy cabbage aphis, *Brevicoryne*

brassicae, L., and it was felt that further observations should be made to determine whether insecticides such as DDT and BHC can be destructive to Aphid parasites and predators and, if so, whether this is sufficient to influence Aphid development. The work described below consists of laboratory, glasshouse and insectary experiments carried out whenever insect material was available during 1945 and 1946. In several experiments the conditions approximated to those of the field and some data were obtained on effects of insecticides on host-predator relationships.

In general only small numbers of insects were available at any time and thus detailed quantitative experiments were not possible.

Effect on certain Syrphids and their Hosts.

Syrphid adults feed on nectar and pollen, and when visiting treated blossom they are liable to be affected by both contact and stomach insecticides whereas on foliage stomach poisoning is unlikely. The toxicity of DDT and BHC on treated foliage and blossom was shown by the following experiments.

BHC P530 spray at about $\frac{1}{2}$ field strength (0·013 per cent. w/v γ-isomer) was sprayed on the upper leaf surfaces of a potted " January King " cabbage plant, the lower surfaces of which were heavily infested with the Aphid, *Myzus persicae*. It was felt that this treatment would be comparable to that occurring in the field. Potted plants were similarly treated with field strengths of DDT Guesarol E spray (0·2 per cent. w/v DDT) and Guesarol dust (5 per cent. w/w DDT).

As in all the following experiments, spraying was carried out by means of an Aerograph M.P. spray gun and dusting by a small hand duster.

The sprayed plants and an unsprayed control plant were enclosed individually in 18 in. × 18 in. × 12 in. muslin cages and fourteen 2–3 day-old adult Syrphids, reared from larvae in the laboratory, were placed in each cage. These were mainly *Syrphus ribesii*, L., with some *S. luniger*, L., and *Catabomba pyrastri*, L.

A bunch of untreated mustard blossom and cotton wool soaked with honey syrup provided food for the adults.

At the same time a similar experiment was set up in which a bunch of open white mustard blossom, *Brassica alba*, was dusted with 5 per cent. w/w Guesarol dust. Another bunch was untreated and each was placed in a 24 in. × 23 in. × 20 in. muslin cage with 20 adult Syrphids from the same stock as above. Cotton wool soaked in honey syrup was provided. The experiments were carried out in the glasshouse under warm sunny conditions.

TABLE I.

The kill of adult Syrphids on cabbage foliage.

Treatment on 16.vii.46	No. of insects	No. of insects dead		Per cent. kill third day
		17.vii.46	19.vii.46	
0·013 per cent w/v BHC P530 spray ...	14	14	—	100
0·2 ,, ,, w/v DDT Guesarol E spray ...	14	0	2	14
5 ,, ,, w/w DDT Guesarol dust ...	14	1	5	43
Untreated control	14	0	0	0

Table I shows the daily kills caused by DDT and BHC treatments on cabbage and clearly indicates that Syrphid adults are highly susceptible to BHC. Several were moribund three or four hours after the beginning of the experiment and all were dead in less than 24 hours. Toxic effects were also shown by DDT, particularly

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by the 5 per cent. dust. DDT had no effect on the *Myzus persicae* host and Aphids were observed to migrate on to the treated areas and reproduce normally. In the BHC treatment it was noticed that the *M. persicae* colonies fell from the treated foliage and died, in spite of the fact that these were situated on the under-surface of leaves, of which only the upper surfaces were sprayed with insecticide. It seems that either BHC was absorbed by the leaf and acted as a stomach poison to *M. persicae* or the insecticide was exerting a fumigant action, which under glasshouse conditions may possibly be exaggerated.

Table II shows the percentage kill of adult Syrphids caused by 5 per cent. DDT dust on mustard blossom. This is greater than that obtained in various experiments using treated foliage but the techniques used were not sufficiently quantitative for it to be possible to state that the difference is significant.

TABLE II.

The kill of adult Syrphids caused by 5 per cent. DDT dust on open mustard blossom.

Treatment on 16.vii.46	No. of insects	No. of insects dead			Per cent. kill third day
		17.vii.46	18.vii.46	19.vii.46	
5 per cent. w/w DDT Guesarol dust ...	20	5	8	2	75
Untreated control	20	1	0	0	5

No repellency was observed in any of the DDT or BHC treatments. In the DDT treatments careful note was made of the time of death of different Syrphid species but no obvious differences in susceptibility were noticed.

Clearly both DDT and BHC, by destroying adult Syrphids, are liable to favour Aphid pests but in the case of the latter insecticide this is offset by its high toxicity to Aphids. The former required further detailed investigation and an experiment was planned to give information, not only on the direct effect of different DDT preparations on adult Syrphids, but also on resultant effect on Aphid colonies (in this case *Brevicoryne brassicae*) which were subject to attack by Syrphids.

One medium sized " Primo " cabbage in a 7-in. pot was carefully sprayed or dusted with each of the following preparations in such a way that complete coverage by a thin and as far as possible, even film of insecticide was obtained.

(1) Sprayed with 0·2 per cent. w/v DDT with Guesarol E base.

(2) Dusted with 5 per cent. w/w DDT Guesarol dust.

(3) Sprayed with 0·1 per cent. w/v DDT suspension.*

(4) Sprayed with 0·01 per cent. w/v DDT suspension.*

(5) Unsprayed control.

After treatment, all plants were infected with *B. brassicae* by placing on them strips of foliage bearing colonies of the Aphid. The Aphids migrated on to the fresh plants and the strips of foliage were removed the following day.

Each plant was placed individually in a 24 in. × 23 in. × 20 in. muslin cage, with white mustard blossom and cotton wool soaked in honey syrup as food.

*Preparation of this suspension is described by McIntosh (1947). The DDT was in the form of flat hexagonal plates 60 × 15µ in a medium containing 0·1 per cent. w/v sulphonated lorol and 10 per cent. v/v acetone.

Adult Syrphids were collected from a cabbage plot in the neighbourhood and twenty placed in each cage. The great majority were *Syrphus* spp.—*S. ribesii*, L., and *S. luniger*, Mg., with a few *Sphaerophoria* spp. and *Eristalis* spp. The plants and cages were examined daily and the numbers of dead adult Syrphids counted at each examination. Plants were examined for Syrphid eggs and the development of the *B. brassicae* cultures was noted.

None of the DDT treated plants was repellent to the adult Syrphids and, at the beginning of the experiment, they alighted on them as frequently as on the control plants. An examination after 16 hours, however, showed that adults in the treatment cages, although not showing typical DDT symptoms, were less active than those in the control, particularly so in cages containing plants treated with the 0·1 per cent. suspension and 5 per cent. dust.

Table III shows the number of adult Syrphids found dead at each examination. The high toxicity of the 0·1 per cent. crystalline suspension is noticeable and there is little doubt that DDT in this form is inherently more toxic to Syrphids. Determination of the amount of DDT retained by each treatment was not made but it was clear that the Guesarol E preparation retained a thicker spray film than the laboratory suspension and thus the toxicity of the latter cannot be due to greater retention.

It was noticed that the *Eristalis* species were the first to be affected by DDT and they all died in the treatment cages within the first two days.†

TABLE III.

The kill of adult Syrphids on treated cabbage foliage.

Treatment on 3.viii.45	No. of insects	No. of insects dead			Per cent. kill third day
		4.viii.45	5.viii.45	6.viii.45	
0·01 per cent. DDT crystalline suspension	20	0	3	0	15
0·1 per cent. DDT suspension ...	20	1	13	4	90
0·2 ,, ,, DDT Guesarol E spray	20	0	5	1	30
5 ,, ,, Guesarol dust	20	1	9	1	55
Untreated control	20	1	0	1	10

Four days after the beginning of the experiment detailed above, egg batches each consisting of 2–3 eggs were found on the underside of outside leaves of the untreated plant. Three days later 15 egg batches were counted from some of which larvae were emerging. No eggs were found on the treated plants, though a larva discovered later on the 0·01 per cent. DDT treatment indicated that at least one egg was laid.

Table IV shows the development of *B. brassicae* during the period of the experiment. The Aphids developed most rapidly on the untreated control plant and attacked all leaves including those in the heart. On the plants sprayed with 0·01 per cent. DDT crystalline suspension and 0·2 per cent. DDT Guesarol E, they also developed well though not on the heart leaves. In spite of several re-infections no Aphid colonies would develop on the plant treated with the 0·1 per cent. DDT crystalline suspension.

The almost complete destruction of Aphids on the control plant between 23rd August, 1945, and 26th August, 1945, was very noticeable. This was caused by Syrphid larvae. A week later the Aphids were still developing well on the Guesarol E sprayed plant, attacking the heart leaves and causing obvious damage.

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†These species are not of economic importance except as pollinators.

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TABLE IV.

Effect of different treatments on development of *Brevicoryne brassicae*.

Treatment	Condition of *B. brassicae* population									
	3.viii.45	5.viii.45	7.viii.45	9.viii.45	13.viii.45	17.viii.45	23.viii.45	26.viii.45	4.ix.45	12.ix.45
0·01% DDT crystalline suspension	plants infested with *B. brassicae*	++	+++	+++	+++	+++	+++	+++	+++	+++
0·1% DDT crystalline suspension		0	Reinfested with *B. brassicae*	+	+ (winged forms only)	0	0	0	Reinfested with *B. brassicae*	++ (one small colony)
0·2% DDT Guesarol E. spray		++		+++	++++	+++++	+++++	++++++	++++++	+++++++
5% DDT Guesarol dust		+		+	++ (one small colony)	++	++	++ (three colonies)	++	+++
Untreated control ...		++	+++	++++	++++	++++++	+++++++	+	0	++ (two small colonies)

+ { Few aphids—not reproducing.
++ { Few aphids—reproducing.

+++ { Colonies well established
++++ { reproducing well

+++++ { Heavy infestation causing
++++++ { obvious damage to plants.

Previous observations indicated that Syrphid larvae were highly resistant to DDT. These were confirmed by three series of laboratory trials and data were also obtained on effects of BHC and other insecticides. In the first experiment Syrphid larvae (*Syrphus ribesii, S. luniger, Catabomba pyrastri* and several unknown species) of varying age were collected from bean plants in the field, randomised and divided into batches of ten. Each batch was placed in a 9 cm. petri dish with Whatman No. 1 filter paper base and heavily sprayed or dusted with the appropriate insecticide. Larvae were then removed to clean petri dishes, placed in a constant temperature cabinet at 75°F., given fresh food daily and kept until pupation, observations being made at daily intervals. No kill was recorded by 0·5 per cent. w/v DDT Guesarol E spray (2×field strength), 5 per cent. w/w DDT Guesarol dust (field strength) or 0·01 per cent. Rotenone in Derris soap spray (2×field strength) ; BHC at ½ field strength (0·013 per cent. w/v γ-isomer) killed four young larvae in the two days following treatment. There was no further kill amongst other BHC treated larvae and pupation occurred normally.

A more detailed experiment was carried out to determine the film effect of various insecticides on Syrphid larvae reared until pupation in continuous contact with surfaces treated with the insecticide.

The insect material was obtained as in the previous experiment, and was divided into batches of about fifteen.

Insecticide films were obtained by treating petri dishes containing a Whatman no. 1 filter paper with 4 ccs. of spray or known weights of dust. The sprays were allowed to dry, batches of larvae together with Bean Aphis for food were placed in each dish and the whole covered with a bolting silk top. Fresh Aphids were provided and examinations made at intervals until pupation.

The experiment was carried out in a constant temperature cabinet at 75°F.

Table V shows the kills recorded after 12 days when practically all larvae had pupated.

TABLE V.

The kill of Syrphid larvae recorded by insecticide films on filter paper.

Insecticide	Conc. of active material in insecticide	Weight in mg. of active material per sq. cm. of film	Proportion of larvae dead after 12 days
Guesarol E. spray ...	0·2% w/v DDT	0·13 mg. DDT	1/15
	0·5% w/v DDT	0·32 mg. DDT	1/13
DDT 60 × 15µ crystal-line suspension ...	0·1% w/v DDT	0·065 mg. DDT	0/17
	0·2% w/v DDT	0·13 mg. DDT	0/19
Guesarol dust ...	5% w/w DDT	0·08 mg. DDT	0/13
		0·4 mg. DDT	3/13
		Dust base control 8 mg.	2/15
BHC P530 spray ...	0·00026% w/v γ-isomer	0·00016 mg. γ-isomer	1/17
	0·0026% w/v γ-isomer	0·0016 mg. γ-isomer	2/15
	0·026% w/v γ-isomer	0·016 mg. γ-isomer	11/15
		Spray base control 0·24 mg.	1/15
PP Flea Beetle dust	0·2% w/w γ-isomer	0·00016 mg. γ-isomer	1/16
Derris soap spray ...	0·0004% Rotenone	0·00026 mg. Rotenone	0/14
	0·004% Rotenone	0·0026 mg. Rotenone	1/16
	0·04% Rotenone	0·026 mg. Rotenone	1/15
Untreated control			0/16

The only significant kill was by the heaviest deposit of BHC P530 spray—
0·016 mg. of γ-BHC per sq. cm. Such a heavy deposit is unlikely to occur under
field conditions though it should be made clear that in experiments with insecticide
films the type of surface treated has an important effect on toxicity of the film
(Potter, 1941 ; Parkin and Hewlett, 1946) and a treated filter paper surface such as
was used in this experiment is probably less toxic than a similarly treated leaf surface.

Conditions comparable with those in the field were obtained in a further experiment
with Syrphid larvae in which data were obtained on the effect of the insecticide on
host-predator relationship.

Plants of field bean bearing a heavy population of Bean Aphis and Syrphid
larvae were collected from the field and placed in pots of water in an outside insectary.
The numbers of Syrphid larvae per batch of plants were counted and, where necessary,
made up to 12–15.

Thorough applications of various insecticides to the potted plants were made
with an Aerograph M.P. hand sprayer and a small hand duster and the plants were
then enclosed individually in open cages in which a sticky band was used to prevent
escape of insects. Examination of the condition of Aphids and Syrphids was made
after two days. Further detailed examinations were not made since, in treatments
where the Aphid host was destroyed, the Syrphids were leaving the plant in search
of further food.

Table VI shows the kill of Syrphid larvae and the condition of the Aphid
population two days after treatment with various insecticides. Kills of Syrphid
larvae were insignificant except in the BHC spray treatments, where a field
concentration of P530 spray killed the younger larvae while 2 × field concentration
was lethal to all but the fully grown larvae of *C. pyrastri*. The order of effectiveness
on the *A. fabae* population was rotenone > nicotine = BHC > DDT.

Results would suggest that rotenone and nicotine act as effective selective poisons
whilst BHC may show similar beneficial effects since, although it can be destructive

TABLE VI.

The kill of Syrphid larvae and conditions of Bean Aphis populations two days after treatment
with various insecticides.

Treatment	No. of Syrphid larvae killed	Condition of Aphids
0·2% w/v DDT in Guesarol E spray	2/16	Majority destroyed. Remainder reproducing normally.
5% w/w DDT in Guesarol dust ...	0/16	Slightly reduced. Large numbers remaining and reproducing normally.
0·013% w/v γ-BHC in P530 spray	8/14	Most destroyed. Remainder reproducing normally.
0·026% w/v γ-BHC in P530 spray...	6/12	Most destroyed. Remainder reproducing normally.
0·052% w/v γ-BHC in P530 spray...	14/17	Completely destroyed.
0·2% w/w γ-BHC in PP Flea Beetle dust	2/13	Most destroyed. Few remaining—reproducing normally.
0·005% w/v Rotenone in derris soap spray	1/14	Completely destroyed.
3% w/w Nicotine dust	2/14	Practically all destroyed. Few remaining—not reproducing.
0·06% v/v Nicotine spray with 0·1% w/v sulphonated lorol wetter	2/17	Completely destroyed.
Untreated control	2/23	Heavy infestation.

to Syrphid larvae, it is clearly highly toxic to Aphids. Syrphid larvae appear to be completely unaffected by DDT, and in fact their resistance to insecticides as a whole is remarkable.

Effect on certain Coccinellids and their Hosts.

Experiments were carried out under similar conditions with Coccinellids and comparative data on the effect of various insecticides on adults, eggs and larvae were obtained. Unsatisfactory oviposition, destruction of eggs by the adults, and cannibalism amongst larvae resulted in the failure of more detailed experiments.

Two experiments with Coccinellid adults are recorded below, the first being a comparison of toxicities of various insecticides, the second a comparison of different DDT preparations.

Batches of 15 *Coccinella septempunctata* adults, collected from various crops in the field, were heavily sprayed and dusted with the insecticides, and the batches enclosed individually in 18 ins. × 18 ins. × 12 ins. muslin cages together with potted " January King " cabbage plants previously treated with the appropriate insecticides. Careful examinations showed, within a few hours, initial signs of effect by the treatments with DDT (staggering and regurgitation), BHC spray (staggering) and rotenone (paralysis). By the 4th day all living BHC and rotenone-treated insects were apparently normal but most of those in the DDT treatments still showed some signs of effect. Table VII shows the kills recorded in this experiment.

TABLE VII.

The kill of adult *C. septempunctata* caused by direct spraying and dusting with various commercial insecticides.

Treatment on 20.viii.46	No. of insects dead			24.viii.46 Total number of dead *C. septempunctata*	24.viii.46 % kill
	21.viii.46	22.viii.46	24.viii.46		
0·2% w/v DDT Guesarol E spray	2	2	0	4/16	25
5% w/w DDT Guesarol dust ...	3	4	1	8/18	44
0·026% w/v γ-BHC P530 spray ...	1	5	3	9/18	50
0·052% w/v γ-BHC P530 spray ...	2	4	1	7/16	44
0·005% w/v Rotenone in Derris soap spray	1	0	0	1/15	7
0·01% w/v Rotenone in Derris soap spray	3	2	1	7/14	50
0·06% v/v Nicotine with 0·1% sulphonated lorol wetter ...	0	0	0	0/15	0
Water sprayed control	0	0	0	0/15	0

The second experiment was planned to give information on host-predator relationships in the presence of DDT. However, at this time of the year—22nd August, 1945—reproduction had ceased and data were only obtained on direct toxicity to adults and to the Aphid host—*B. brassicae.*

The experiment was carried out in the glasshouse using the same technique as that described for the Syrphid experiment on page 281. Treatments were similar, " Primo " cabbage plants being sprayed and dusted with DDT and afterwards infested with *B. brassicae.* Plants were caged individually and on the day after spraying thirty adult *C. septempunctata*, collected six days previously from Aphid-infested cabbages in the field, were placed in each cage.

During the period of the experiment the weather was mainly dull and the Coccinellids remained rather inactive ; they tended to leave the plants and cling to

TABLE VIII.

Effect of DDT-treated cabbage foliage on adult *C. septempunctata* and *B. brassicae*.

Treatment on 22.viii.45		Date					Total no. of dead *C. septempunctata* 5.ix.45	% kill 5.ix.45
		23.viii.45	24.viii.45	26.viii.45	29.viii.45	5.ix.45		
0·01% w/v DDT 60 × 15μ crystalline suspension	No. of dead *C. septempunctata*	0	0	0	0	0	0/25	0
	Condition of Aphids	+	++	+++	+++	+++		
0·1% w/v DDT 60 × 15μ crystalline suspension	No. of dead *C. septempunctata*	4	3	0	0	0	7/25	28
	Condition of Aphids	+	+	+	None	None		
0·2% w/v DDT Guesarol E spray	No. of dead *C. septempunctata*	8	6	2	2	4	22/26	85
	Condition of Aphids	+	++	++	++	+++		
5% DDT Guesarol dust	No. of dead *C. septempunctata*	6	1	8	3	10	28/28	100
	Condition of Aphids	+	+	+	None	None		
Untreated control	No. of dead *C. septempunctata*	0	0	0	0	0	0/24	0
	Condition of Aphids	+	++	+++	+++	+++		

the muslin of the cages in both treatments and control. Variations in Aphid population on different treatments which would affect the attractive properties of the plants to the Coccinellids made it impossible to determine whether any treatments were repellent.

Examinations were made at intervals and Table VIII shows the kills of adult *C. septempunctata* and the condition of the *B. brassicae* population at each examination.

The high kill caused by 0·2 per cent. w/v DDT in Guesarol E spray and the low kill by 0·1 per cent. w/v DDT crystalline suspension is worthy of note. This contrasts with the results of the similar experiment with adult Syrphids (Table III) in which the reverse was the case.

The dull weather and possible destruction by Coccinellids* were the main causes of rather poor development of the *B. brassicae*. As in the Syrphid experiment the 0·1 per cent. crystalline suspension and 5 per cent. dust prevented development of the Aphids.

Observations on small numbers of *Adalia bipunctata* showed that this insect had a relative resistance to insecticides comparable with that of *C. septempunctata*, but the speed of toxic action was apparently rather more rapid.

An experiment was carried out to determine the effect of certain insecticides sprayed directly on eggs of *C. septempunctata* and *A. bipunctata* in order to provide information on ovicidal action and action on newly emerged larvae under conditions which approximate to those of the field.

Field bean leaves bearing egg batches of *C. septempunctata* and *A. bipunctata* were collected from the field and carefully sprayed with various insecticides by means of an Aerograph M.P. paint sprayer. The number of eggs per batch was counted, and, as far as possible, two batches treated with each insecticide used. The treated leaves were placed individually in phials of water in small muslin cages, together with unsprayed leaves bearing Bean Aphis as food for the newly emerged larvae.

The time of hatching was noted and counts made of the numbers of larvae on the two days following emergence, by the end of which time the larvae were either dead or normal and feeding voraciously on the Bean Aphis. The experiment was carried out in an outdoor insectary.

Results are not clear cut for two reasons : (1) about $\frac{1}{3}$ of the eggs in a batch were destroyed by those larvae which emerged first and consumed some of the unhatched eggs around them, (2) larvae which died soon after emergence quickly shrivelled and sometimes could not be found and consequently the actual kill was difficult to determine.

Table IX shows the percentage kills by the various treatments in which correction has been made for natural mortality, based on kill or losses in the controls. Kills approaching 100 per cent. indicate toxicity but where low kills are recorded they are of doubtful significance.

The results show clearly that rotenone even at half field concentration (0·0025 per cent. w/v) has an ovicidal action, the eggs developing to a certain stage becoming pigmented and apparently dying just prior to emergence though in many cases half emerged larvae died. The DDT and γ-BHC sprays were not ovicidal but field concentrations showed toxicity to larvae after emergence. A BHC dust containing 0·2 per cent. w/w γ-BHC was harmless as was a nicotine spray. From this and further small experiments it was clear that eggs of *A. bipunctata* and *C. septempunctata* showed a similar level of resistance to insecticides. It was felt that the age of the egg at the time of treatment might be important in relation to its resistance but a sufficient number of eggs was not available at the time for carrying out more detailed experiments.

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*No adult Coccinellids were observed feeding.

TABLE IX.

Toxicity of various insecticides when sprayed on Coccinellid eggs.

Treatment	Batch	No. of eggs	Days after treatmeat when emergence occurred	Larvae alive two days after emergence	% kill after two days corrected for control	Notes
0·05% w/v DDT Guesarol E spray	a	55	4	31	10	
	b	52	5	30	10	
0·2% w/v DDT Guesaiol E spray	a	55	5	14	60	
	b	48	6	0	100	
0·013% w/v γ-BHC P530 spray	a	48	2	28	5	
	b	56	2	25	25	
0·026% γ-BHC P530 spray		50	4	12	60	
0·052% γ-BHC P530 spray	a	30	2	0	100	
	b	36	3	0	100	
0·2% w/w γ-BHC PP Flea Beetle dust		50	5	31	0	
0·0025% w/v Rotenone derris soap spray	a	45	3	11	60	20 died during emergence.
		38	7	0	100	1 emerged and died, rest killed in egg.
0·005% w/v Rotenone derris soap spray	a	64	5	4	90	Many killed in egg — others died when half emerged.
	b	24	3	0	100	
0·01% w/v Rotenone derris soap spray		45	—	0	100	All killed during egg stage.
0·06% v/v Nicotine, 0·1% sulphonated lorol wetter		42	2	30	0	
Unsprayed controls ...	a	45	5	24 ⎫	0	
	b	38	3	27 ⎭		

Small numbers of larvae were used in preliminary experiments to determine the effect of insecticides on Coccinellid larvae. A technique similar to that of the Syrphid larvae experiment described on page 285 was adopted.

Half grown larvae of *C. septempunctata*, reared in the glasshouse on Bean Aphis, were divided into batches of five and direct-sprayed in 9 cm. petri dishes (Whatman No. 1 filter paper base) with various insecticides. They were then transferred to clean dishes (no filter paper base) and provided with numerous Bean Aphids as food. It is especially important that the food supply is adequate since cannibalism occurs whenever food is not readily available and also where overcrowding occurs.

Table X shows the kill recorded after two days.

Data show that the larvae were very susceptible to the field concentration of the Derris spray and were also affected by the DDT spray whereas a field concentration of the BHC spray showed only slight toxicity and caused a kill which may be of little significance. Results indicating the greater toxicity of the DDT preparation compared with the BHC spray at field strengths were confirmed in further small trials.

TABLE X.

Toxicity of various insecticides as direct contact sprays on *C. septempunctata* larvae.

Treatment	No. of larvae dead after 2 days
0·05% w/v DDT Guesarol E spray ...	0/10
0·2% w/v DDT Guesarol E spray ...	4/10
0·013% w/v γ-BHC P530 spray	0/11
0·026% w/v γ-BHC P530 spray	2/10
0·005% w/v Rotenone derris soap spray...	11/11
0·0005% w/v Rotenone derris soap spray	1/10
Unsprayed control	0/11

Effect on certain Braconids and their Hosts.

Preliminary experiments were carried out with certain parasites of *B. brassicae* and *M. persicae* but the results were not wholly satisfactory because of the difficulties of maintaining the adult parasites under laboratory conditions.

Adult *Aphidius brassicae*, Marsh., and *Aphidius matricariae*, Hal., lived satisfactorily in small cages but in experiments where cages as large as 1 ft. × 1 ft. × 9 ins. were used, the natural mortality was always abnormally high. For this reason experiments to determine the extent of Aphid control by parasites in the presence of insecticides produced insignificant results. The following results are, however, worthy of mention : —

Aphidius brassicae.—Adults were collected as they emerged from parasitised *B. brassicae* and kept in 3 ins. × 1 in. glass tubes, with honey syrup as food, for one to two days before enclosure in small cages with treated cabbage plants. The design of the cages is important. For experiments with DDT a cellophane bag inverted over a small cabbage plant in a 5 ins. pot was used. The soil in the pot was covered with a layer of silver sand which enabled dead parasites to be observed and counted. With BHC treatments the cellophane was replaced by bolting silk in order to eliminate abnormal fumigant effects.

Table XI shows the daily kills recorded when twenty *A. brassicae* adults were enclosed as above with DDT-treated cabbage plants. No signs of repellency were observed.

TABLE XI.

The kill of adult *A. brassicae* enclosed in cages with DDT treated cabbage plants.

Treatment on 24.ix.45	No. of insects dead at each examination				Per cent. kill 4th day
	25.ix.45	26.ix.45	27.ix.45	28.ix.45	
0·1% w/v DDT 60 × 15μ crystalline suspension ...	7	9	4	—	100
0·2% w/v DDT Guesarol E spray	2	4	1	5	57
Untreated control	0	1	1	2	22

Experiments with BHC showed that an application of BHC P530 spray at 0·013 per cent. w/v γ-BHC (half field strength) and flea beetle dust (0·2 per cent. w/w γ-BHC) to foliage caused 100 per cent. kill of *A. brassicae* adults within a few hours. Field concentrations of the spray on turnip also caused complete destruction of the Aphid host *B. brassicae*.

Aphidius matricariae.—Several experiments were carried out during 1946 in an attempt to determine the effect of insecticides on host-parasite relationships and for these I am particularly indebted to Miss J. Bathgate for much careful work in the rearing and treatment of the parasites. As mentioned before the results were inconclusive except that in experiments with BHC the following data were obtained :—

(1) *A. matricariae* adults were all destroyed within a few hours when enclosed in a bolting silk cage with a turnip plant treated with 0·026 per cent. γ-BHC in P530 spray.

(2) A plant treated as above and kept in the glasshouse showed toxicity to the parasite adult for at least three weeks after treatment.

(3) *Myzus persicae*, the host of *A. matricariae*, was highly susceptible to BHC. Complete destruction of Aphids feeding on the lower surface was obtained by spraying the upper surface of turnip leaves with 0·026 per cent. γ-BHC in P530 spray.

Effect on certain Cecidomyiids and their Hosts.

Preliminary experiments were carried out with larvae (probably of a species of *Phaenobremia*) on *Macrosiphum urticae*, Schr. *Phaenobremia* spp. are recorded as predators of *B. brassicae* and *M. persicae* (Barnes, 1929, Petherbridge & Wright, 1938).

Leaves of *Urtica dioica* (Stinging Nettle), bearing half to fully grown larvae were sprayed with DDT and BHC preparations and placed in an outdoor insectary. The larvae were observed for the next three days and found to be susceptible to both DDT and BHC. An 0·1 per cent. w/v DDT preparation of $60 \times 15\mu$ crystals caused typical DDT symptoms—continuous rippling movements of the insect body wall, shrivelling and finally death. Several larvae treated with 0·2 per cent. DDT in Guesarol E showed similar symptoms. Five per cent. w/w DDT dust adhered strongly to the insect body wall but although deaths resulted, the typical DDT effects were not noticed. The data in Table XII show clearly that the dust base was toxic. Both BHC P530 spray and PP Flea Beetle dust caused a kill ; continuous wriggling movements, suggestive of γ-BHC poisoning being observed among the larvae in both treatments.

Table XII shows the condition of the larvae three days after treatment by the various insecticides.

TABLE XII.

Condition of larval Cecidomyiidae three days after direct spraying and dusting with DDT and BHC insecticides.

Treatment	No. of insects	No. dead	No. affected	No. normal	Notes
0·2% w/v DDT in Guesarol E spray	10	3	2	5	DDT symptoms noticeable in some larvae
5% w/v DDT in Guesarol dust	9	5	4	0	No DDT symptoms noticeable. Insects quiescent and shrunken
Guesarol dust base ...	8	5	3	0	Quiescent—shrunken
0·1% 60 × 15μ DDT spray with 0·1% Sulphonated lorol wetter	9	4	3	2	DDT symptoms noticeable in some larvae
0·026% w/v γ-BHC in P530 spray	9	6	3	0	γ-BHC symptoms noticeable in most larvae
0·2% w/w γ-BHC in PP Flea beetle dust	8	8	–	0	γ-BHC symptoms noticeable in most larvae
Untreated control ...	8	1	1	6	In general remained quiescent

Discussion.

Table XIII indicates the relative toxicities of insecticides at field strength to several predatory and parasitic insects, and to their Aphid hosts, based on the results of the laboratory and glasshouse experiments.

TABLE XIII.

Relative toxicities of insecticides at field strength to several predators and parasitic insects and to Aphids.

	DDT	γ-BHC	Rotenone	Nicotine
Syrphidae				
Adults	+++	++++	—	—
Larvae	Unaffected	+	Unaffected	Unaffected
Coccinellidae				
Adults	+++	++	++	Unaffected
Larvae	+++	++	++++	Unaffected
Eggs	Unaffected	Unaffected	++++	Unaffected
Braconidae (*Aphidius* spp.)				
Adults	+++	++++	—	—
Cecidomyiidae (*Phaenobremia* spp.)				
Larvae	+++	+++	—	—
Aphidae				
Apterae	++	+++	++++	++++

+ Only a proportion of insects affected or destroyed.
++ Varying number unaffected.
+++ All insects either affected or destroyed in time.
++++ Rapid destruction of practically 100% of insects.

The data provide further confirmation of the fact that nicotine acts as a selective insecticide in that Syrphid larvae and Coccinellids were unaffected at the concentrations used whilst the Aphid host was highly susceptible.

BHC showed toxic properties to all the beneficial species tested. Adult Braconid and Syrphid species were highly susceptible and showed no signs of being repelled from treated foliage whilst Syrphid larvae and Coccinellids were only slightly affected. Clearly the toxicity of BHC to Aphids must help to counterbalance the danger that may result from its destruction of Aphid predators and parasites, though under field conditions sedentary Aphids which are often found in situations protected from insecticidal action are less likely to come into contact with treated surfaces than are the free living forms of entomophagous insects. Thus, there may be selective destruction of beneficial insects in spite of an inherently high susceptibility of Aphids.

The conclusion drawn from the present work is that certain entomophagous insects are susceptible to BHC and that as a result there is a possibility that the use of this insecticide in the field may favour the development of Aphids in spite of the fact that the latter are themselves vulnerable.

DDT in the preparations used was toxic to most entomophagous species. Aphids were also affected, though in most cases this took the form of a temporary check in the development of the colonies. DDT inhibited egg-laying by adult Syrphids and in one experiment it was shown that a cabbage plant treated with 0·2 per cent. DDT Guesarol E spray suffered greater damage from *B. brassicae* than an untreated control plant because of inhibition of egg-laying by Syrphid adults and a consequent absence of larvae.

DDT was less toxic than BHC to adult and larval Syrphids, larval Cecidomyiids and adult Braconids. Its relatively slight effect on Aphids, at any rate in certain

preparations, suggests that in the field its use may be more favourable to the development of Aphids than BHC.

The above conclusions regarding relative toxicities of DDT and BHC are based on results of experiments in which only certain specific insecticidal preparations were used. Consequently only general conclusions may be drawn from them since the level of toxicity of an insecticide to any particular insect may vary considerably according to wetting, spreading and penetrating properties of the insecticide medium. DDT is at present finding its chief use in the control of various lepidopterous and coleopterous pests of orchard and field crops. Against such pests a persistent protective film is required. Efficient wetting and spreading materials in the spray medium are disadvantageous since these result in greater "run off" and poor retention of insecticide, thus reducing persistence. For this reason dusts and wettable powder sprays with poor wetting and spreading properties are generally used. Such preparations are not satisfactory for the control of Aphids, which require sprays with good wetting and spreading properties, but they retain toxicity to the Aphid predators and parasites. Thus in the present work 0·2 per cent. w/v DDT in Guesarol E spray caused only temporary reduction in the development of Aphid populations but showed greater toxicity to adult *C. septempunctata* than that of an 0·1 per cent. w/v laboratory preparation with good wetting properties, which appeared to be persistently toxic to Aphids but had little effect on the adult Coccinellid. Such data provide additional evidence for the necessity for fundamental studies of the effects of the medium in insecticidal preparations.

The picture is thus highly complicated. In the field in any one year many factors are involved in the host-predator-insecticide relationship : abundance of host, abundance of various species of parasite and predator, nature and mode of preparation and time of application of insecticide, type of crop, and general climatic conditions, to mention only a few. Clearly the evidence that insecticides such as DDT encourage development of pests by destruction of their parasites and predators, although proven in the case of red spider, requires to be substantiated by long-term observations on insect populations in the field. Massee (private communication) and Hough & others, 1945, have made important observations that although beneficial insects were destroyed following application of DDT in the field, their numbers increased rapidly when treatment was discontinued, but whether widespread and long-term use of insecticides is liable to deplete parasite populations permanently is not known. The problem is important in view of the development of resistant strains of insect pests and their artificial selection by insecticides (Hough, 1943, Quayle, 1943, Ripper, 1944). Entomophagous insects, if present, may play an important part in the destruction of such resistant individuals whenever they appear in the pest population.

Summary.

Experiments were carried out during 1945 and 1946 to determine the effect of insecticides, particularly DDT and BHC, on certain species of Syrphids and Coccinellids. In addition, some preliminary data was obtained on toxicity to parasitic Hymenoptera and a predatory Cecidomyiid species.

In some experiments other insecticides were used for comparative purposes and where possible data were obtained on the effect of the insecticide not only on the predator but also on the host and the host-predator relationship.

Syrphidae.

DDT was not repellent but was destructive to alighting adult Syrphids when sprayed on cabbage foliage. An 0·1 per cent. w/v DDT suspension of $60 \times 15\mu$ crystals showed a toxicity comparable to that of a 5 per cent. w/w DDT Guesarol dust and greater than that of an 0·2 per cent. DDT Guesarol E spray. Egg laying

was inhibited even by a sub-lethal DDT concentration. Apterae of the Aphid host, *Brevicoryne brassicae*, placed on treated foliage were not seriously affected by the commercial DDT preparations but on foliage treated with an 0·1 per cent. crystalline suspension of DDT the Aphids did not reproduce and died within 1–3 days.

Under the same conditions a field concentration of P530 spray (0·026 per cent. w/v γ-BHC) on cabbage caused 100 per cent. kill of adult Syrphids within 24 hours and also destroyed apterous females of the host *B. brassicae*.

Syrphid larvae proved to be resistant to field concentrations of DDT (0·2 per cent. spray and 5 per cent. dust), rotenone (0·005 per cent. w/v spray) and nicotine (0·06 per cent. v/v spray). Concentrations of commercial DDT in excess of those used in the field also showed no toxicity. At field strength a P530 spray (0·026 per cent. γ-BHC) was destructive to the young Syrphid larvae but 0·2 per cent. w/w γ-BHC in a commercial Flea Beetle dust had no effect.

Where apterae of *Aphis fabae* were subjected to the same insecticidal treatment as the Syrphids the order of effectiveness of insecticides was Rotenone> Nicotine= γ-BHC> DDT.

Coccinellidae.

DDT on cabbage foliage was destructive to adult *Coccinella septempunctata* and, as in the case of Syrphid adults, different preparations varied in toxicity, the order being 0·2 per cent. w/v DDT Guesarol spray=5 per cent. w/w DDT Guesarol dust >0·1 per cent. w/v DDT crystalline suspension. Field concentrations of a γ-BHC P530 spray and a Derris soap spray also showed some toxicity but both 0·2 per cent. γ-BHC Flea Beetle dust and an 0·06 per cent. v/v nicotine spray were non-toxic. As far as could be observed the treatments showed no repellency.

Derris spray at 1/2 field concentration (0·0025 per cent. w/v Rotenone) had an ovicidal action on the eggs *C. septempunctata* and *Adalia bipunctata*, whilst DDT and BHC sprays caused a kill only after emergence of the larvae. 0·2 per cent. w/w γ-BHC in a Flea Beetle dust and 0·06 per cent. v/v nicotine spray were apparently harmless to both eggs and newly emerged larvae.

Direct spraying of half grown larvae of *C. septempunctata* by field concentrations showed 100 per cent. kill by Derris, and some toxic effects by DDT and BHC sprays.

Braconidae.

Preliminary experiments showed that adults of *Aphidius brassicae* were highly susceptible to BHC and that they were also destroyed by field concentrations of DDT on foliage.

More detailed experiments with adult *Aphidius matricariae* produced inconclusive results because of high losses in the controls two days after treatment. A field concentration (0·026 per cent. w/v γ-isomer) of γ-BHC in P530 spray on turnip foliage caused 100 per cent. kill of adult *A. matricariae* within 24 hours and remained persistently toxic for three weeks. BHC was shown to be highly toxic to apterae of *Myzus persicae* (the host of *A. matricariae*), the fumigant action being sufficient to cause death.

Cecidomyiidae.

Preliminary experiments showed that larvae, probably of a species of *Phaeno-bremia*, were killed by treatment with field concentrations of both DDT and BHC insecticides.

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RECENT WORK ON MERCURY AS AN INSECTICIDE AGAINST INSECT PESTS OF STORED GRAIN.

By M. Maqsud Nasir, M.Sc. (Agric.).

Assistant Entomologist, Quetta.

Mercury is not a new addition to the list of preservatives for stored grain. It has insecticidal properties that are well known to the peasants who insert a drop of mercury in an excavated soap-nut (*Sapindus detergens*) or cowdung ball which is then placed in the stored grain to protect it against the ravages of insect pests. This method of application was considered dangerous, in the case of grain meant for consumption, because the cowdung balls are porous and with a slight jerk mercury droplets get mixed with the grain. A little mishandling in the case of soap-nuts also causes the mercury to escape into the grain heap, thus affecting the health of the consumer adversely. These facts were first recorded by Kunhi Kannan (1920) who initiated the study of the insecticidal properties of mercury on scientific lines by experimenting with Bruchid beetles and silkworms and found that the eggs of these insects, when exposed to mercury vapour, did not hatch but turned black. Larson in the United States (1922) tested mercury vapour against *Bruchus quadrimaculatus*, F., a pest of cowpeas, and confirmed these observations.

Dutt and Puri (1929) carried out investigations at Pusa and recommended the use of mercury in the form of mercury-tin amalgam because it was easy to handle and its vapour pressure being low did not render the grain unfit for seed purposes or human consumption (60 maunds* of grains treated in this manner were used by a family without any untoward effect). The amalgam was prepared by rubbing together 2 parts of tin and 3 parts of mercury into a homogenous mass of paste-like consistency. This mass was then squeezed through a piece of linen, thus removing the free droplets of mercury and eliminating the possibility of mercury becoming mixed with the grain. The amalgam was flattened to expose a greater effective surface and half an ounce was sufficient to keep 14 seers* of wheat free from the attack of *Sitophilus oryzae*, L. Rahman (1942) recommended mercury at the rate of 3–4 tolas* per maund of wheat. He suggested that mercury carried in twill or long-cloth bags measuring 2×2 ins. at the rate of 1 tola of mercury per bag, should be distributed evenly in layers throughout the bin, with a depth of 8 to 12 ins. of wheat between two successive layers. These bags come out easily when the grain is removed through the exit hole and he was of the opinion that this type of application had no deleterious effect on the germination of the seed or its eating quality. The author on the other hand feels that bags are not safe to use because a little mishandling will cause the mercury to escape and render the grain unsafe for consumption.

The Government Entomologist, Punjab, in his Annual Report for the year 1938–39 stated that the use of mercury in bins to control Khapra, *Trogoderma granarium*, Everts, completely checked the attack when it was uniformly distributed in the grain, but, if only the top and bottom portions were treated, the attack continued although at a slower rate than in the control bins. In view of these differences of opinion, an extensive series of experiments was conducted to study the effective range of mercury with the following results :—

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*1 Maund = 82¼ lb.   1 Seer = 2·05 lb.   3 Tolas = 1 oz.

Date	Receptacle		Percentage hatching at different distances in inches							Percentage hatching in control
			7	15	23	31	39	47	55	
18–24/iv/44	A	Airtight	0	0	0	0	€2	85	88	89
		Non-airtight	96	76·5	91·5	78	88·5	95·5	93	89
16/vi/44	B	Airtight	0	0	0	0	54	100	100	100
		Non-airtight	80	84	100	100	97	100	100	100
16–24/vi/44	C	Airtight	0	0	0	0	46	79	92	89
		Non-airtight	48	53	88	92	89	78	95	100

Date	Receptacle		Percentage hatching at different distances in inches					Percentage hatching in control
			0	6	12	24	36	
27/vi–1/vii/44	D_1	Airtight	0	0	0	0	0	100
		Non-airtight	0	87·9	84·1	95	89	95
27/vi–1/vii/44	D_2	Airtight	0	0	0	0	0	91
		Non-airtight	0	41	39	65	62	89
3–10/vii/44	E_1	Airtight	0	0	0	0	0	85
		Non-airtight	5	36	75	42	100	94
14–19/vii/44	E_2	Airtight	0	0	0	0	0	85
		Non-airtight	86	63	100	87	95	88

A. Glass cylinders with mercury placed at bottom.
B. Glass cylinders with mercury placed at top.
C. Glass cylinders placed so as to test effective range of mercury sideways.
D_1. Empty iron bin with mercury placed at bottom.
D_2. Iron bins full of sorghum grains with mercury placed at bottom.
E_1. Empty iron bin with mercury placed at top.
E_2. Iron bins full of sorghum grains with mercury placed at top.

The mercury was placed in the receptacles 15 days before the eggs, pasted on cardboard, were introduced at the various distances.

From the Table above it is clear that mercury vapour is effective only under air-tight conditions and that in glass cylinders measuring 4×8 ins. or iron bins measuring 36×9 ins., the mercury vapour is effective up to a radius of 36 ins. Krishnamurti and Appanna (1945) have made similar experiments and found that the mercury vapour is effective to a distance of 14·5 cm., while A. N. Puri and S. Bharihoki (unpublished) have recently performed experiments in glass tubings with diameter 1·5 to 3 ins. and have shown that the diffusion of mercury vapour is equal in all directions and that the effective range does not extend beyond 37 ins.

Gough (1938) tested the effect of mercury upon larvae of *Tribolium confusum*, Duv., no observations having previously been made on the effect of mercury on stages of insects other than the eggs. He concluded that, while the eggs were affected by the vapour, the other stages were not harmed. These conclusions were not confirmed by Dole (1943) who showed that younger larvae, ⅛ of an inch or less in size, are killed. This author further suggested that copper plates $1 \times 1·5$ in. in size coated with 0·40 grams of mercury and kept in perforated paper boxes were a better substitute for an amalgam because in this form the quantity of mercury required was 1/12th of that used by previous workers. He concluded, in view of Glaser's work (1903) and his own observations, that treated grains were not in any way spoilt by the use

of mercury.   He improved his method further by depositing fine drops of mercury on strips of porous paper in place of mercury coated copper plates.   Wright (1944) tested different forms of mercury, e.g. pure metal, calomel, zinc and tin amalgams, and found that the last three forms were less effective than the first ; he also showed that fine subdivision of the mercury definitely increased its efficiency.   He performed germination and spectroscopic tests of the treated grain and found that the seed had suffered no ill-effects.   Dispersion of mercury in fine drops in cowdung or soil has been known to the farmer and its use on scientific lines was first demonstrated by Dole (1943).   It is the general practice in the North West Frontier Province (Bannu District) to add a mixture of two tolas of mercury, one seer of " Ajwan " (Carum copticum) and 4 seers of cowdung ashes to stored barley.   A part of the mixture is spread at the bottom before the container is filled and layers of the mixture are spread over subsequent grain layers.   Mercury so dispersed is a definite improvement upon amalgams and mercury coated copper plates.   As referred to above, Dole (1943) prepared strips of porous paper upon which fine drops of dispersed mercury were deposited.   This effected an economy of mercury to the extent of 75 per cent. ; about one pound was needed to prepare 300–320 strips and this was sufficient to treat 150 maunds of grain stored in bags.   The strips were tested by the writer in ten pound bags of Andropogon sorghum but did not give very satisfactory results.   It was observed, further, that at pressures of about 67 lbs. and above vigorous shaking in a bottle caused the mercury drops to come out of the paper. This method, therefore, does not seem suitable for treating grain stored in bags kept in piles and meant for consumption.

Experiments were conducted by the author on the use of mercury in materials such as chalk.   Two, four and six grams of mercury were dispersed in 36 grams of chalk and then mixed in 40 lbs. of Jowar (Andropogon sorghum) on 14.ii.44. Fifty adult insects each of Sitophilus oryzae, L., Rhizopertha dominica, F., Tribolium castaneum, Hbst., and 50 eggs each of Corcyra cephalonica, Staint., and Sitotroga cerealella, Ol., were used in each treatment.   Observations were made after 6 months, i.e. on 14.viii.44, when each lot was weighed after sieving out the dust.   There were three replications of each treatment.   The results are presented in the following table :—

No.	Kind of treatment	Weight of grains after six months.
1.	Two grams of mercury dispersed in 36 grams of chalk and mixed in 40 lbs. of " jowar "	(i) 41 lbs. (ii) 41 lbs. 4 ozs. (iii) 41 lbs.
2.	Four grams of mercury dispersed in 36 grams of chalk and mixed in 40 lbs. of " jowar "	(i) 40 lbs. 4 ozs. (ii) 40 lbs. 5 ozs. (iii) 40 lbs. 4 ozs.
3.	Six grams of mercury dispersed in 36 grams of chalk and mixed in 40 lbs. of " jowar "	(i) 39 lbs. 2 ozs. (ii) 41 lbs. (iii) 40 lbs. 2 ozs.
4.	Control	(i) 28 lbs. 13 ozs. (ii) 24 lbs. 6 ozs. (iii) 31 lbs. 11 ozs.

It is quite clear from the above table that the treated grain suffered no loss from insect damage whilst considerable loss in weight occurred in the control.   At the end of the experiment, a few adult Rhizopertha dominica, Sitophilus oryzae and Tribolium castaneum were observed which might have been survivors from the original introduction in the treated grain ;   in the untreated lots all stages of the

insects were seen in very large numbers. It is, therefore, quite apparent that even 2 grams of mercury dispersed in 36 grams of chalk and mixed in 40 lbs. of " Jowar " completely arrested the multiplication of the insects. The increase in weight in the treated lots was due to the increase in moisture content of the grain caused by change in the humidity.

It was suspected that in the above experiments mercury droplets had got mixed with the grain and were not completely removed by sieving out the mercury-chalk powder. The method was, therefore, restricted to the treatment of grain for seed. To overcome this difficulty, slabs were prepared by mixing plaster of paris in mercury-chalk powder which could be fixed to the walls of an airtight storage receptacle. These slabs were tested in glass jars of a capacity of 3,600 cc., against eggs of *C. cephalonica* in large desiccators at 78–82°F. and were found to arrest the development of the eggs. Puri and Bharihoki performed similar experiments and recommend the plastering of the walls of a godown with mercury dispersed in powder. The use of mercury slabs on the walls or the plastering of walls are both impracticable for godowns because the effective range of mercury, as pointed out above, does not extend beyond 37 inches, and consequently this type of application is limited to small storage receptacles. Mercury paper strips (Dole, 1943), mercury slabs or mercury powder packets can be easily and safely used in checking insect infestations in various types of storage receptacles. The only precaution necessary is to effect proper distribution of the material in the light of the effective range of mercury vapour. In a " khatti " the insect attacks are always restricted to the top layers and hence mercury should be distributed only in the upper 2–3-ft. layer. In this connection recommendations made by Rahman (1942) when " khapra " infestations are to be checked, are very valuable. He states that the distribution of mercury in the bins should be carried out in such a way that the upper 10-12-inches layer of grains receives 44–47 per cent. of the total quantity of mercury.

Krishnamurti and Appanna (1945) made a detailed study of the influence of mercury on eggs of *C. cephalonica* and their observations are similar to the results obtained by the author. They observed that the effect of mercury vapour on about 16-hour old eggs, after 48 hours' exposure, was to cause shrinkage and that the vapour entered the embryo through the micropyle of the egg. They also found that 29-hour old eggs of *C. cephalonica* and up to 48-hour old eggs of Bruchids were similarly affected.

Eggs of *C. cephalonica*, pasted on cardboard, were placed by the author in glass desiccators containing mercury and taken out after the desired exposure. The experiment was performed at 78–82°F. with the following results.

Date	The developed stage of the eggs exposed	Exposure to mercury vapours in hours and percentage mortality					Percentage hatching in control
		24	48	72	96	120	
7/viii/44	24 hours after egg laying	20	65	100	100	100	95
	48 hours after egg laying	18	100	100	100		100
	72 hours after egg laying	8	10	3			92
	Freshly laid eggs ...	4	71	100	100	100	93

From the above table, it will be seen that 24-hour old eggs required at least 72 hours' exposure for complete mortality, while 48-hour old eggs were killed after 48 hours' exposure and that 72-hour old eggs remained unaffected and hatched after 24 hours. Freshly laid eggs required at least 72 hours' exposure for complete mortality (temperature 78-82°F.),

The efficiency of mercury vapour on eggs of *C. cephalonica* under varying temperatures and humidities has also been studied by the author. The results showed that a temperature in the range of 20–35·5°C. and humidity from 53–90 per cent. did not reduce the effectiveness of mercury. Puri and Bharihoki, in experiments on the fumigation of grain, succeeded in killing the eggs in a closed space into which air saturated with mercury was drawn. The author made tests by passing air saturated with mercury vapour through a flask of capacity of 300 cc. for 5 minutes with and without grain before sealing and obtained 100 per cent. mortality in the case of eggs of *C. cephalonica*. This method has the disadvantage that, whilst the air saturated with mercury kills the eggs, it has little effect upon mature larvae, pupae and adults. The duration of the various stages lasts for a considerable time and the oviposition period also extends over several months in certain insects. It will not, therefore, be a simple matter to hold the saturated air sufficiently long to allow the immature stages to develop into adults and lay eggs or to judge the correct moment, when oviposition has taken place, to fumigate. The problem becomes all the more difficult where mixed generations are involved.

Summary.

A review of the work carried out on mercury as a preservative against stored grain pests is given.

The influence of the duration of exposure to mercury vapour upon eggs of different stages of development is discussed.

Temperature and humidity within ordinary limits do not affect the efficiency of mercury.

Under airtight conditions this preservative is effective within a radius of three feet in receptables with or without grain.

Paper strips coated with mercury cannot be recommended for grain stored in bags and kept in piles because pressure and weight of the grain, or even shaking, will cause the mercury to be expelled from the strips.

Mercury dispersed in chalk cannot be used for grain destined for consumption. It is, however, quite suitable for preserving grain for seed.

Plastering the walls with mercury paste or affixing slabs to the walls of godowns is not effective owing to the limited range of mercury vapour.

Application of mercury in various types of receptacles is discussed and advocated.

Fumigation with mercury does not seem to be practicable.

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304   M. MAQSUD NASIR.

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HIBERNATION OF *HYALOMMA SAVIGNYI* (IXODIDAE) IN PALESTINE.

By B. FELDMAN-MUHSAM, Ph.D.

Department of Parasitology, Hebrew University, Jerusalem.

Galuzo (1941) in his ecological studies of the tick fauna in Kazakh S.S.R. states that the nymph of *Hyalomma detritum*, Schulze, overwinters on the host, that he is of the opinion that *H. savignyi*, Gerv., hibernates in the adult stage away from the host (in animal quarters or houses) and that he found hibernating nymphs of *H. marginatum*, Koch, in grass meadows.

In the course of field work carried out in Palestine during the winter 1945–46 only a few adult male and female ticks (some gorged) were found in the Jordan Valley and the Negeb, and none in the more temperate parts of the country.

The fact that no larvae or nymphs were found does not exclude the possibility of their presence and, to determine whether they can survive and hibernate at low temperatures, experiments were carried out with *H. savignyi*, the commonest tick in Palestine. These were arranged in three series.

I. In a thermostat at 32°C., and about 80 per cent. R.H.

II. In a cupboard placed in a large unheated corridor, the mean temperature of which was 20°C. in November descending to 16°C. in March and later rising to 22°C. in June and 25°C. in July. The relative humidity ranged between 40 and 50 per cent.

III. Out of doors. All test tubes containing material were put on the outer side of a northern window, the mean monthly temperature of which was 16°C. in November descending to 7·6°C. in February and rising to 20°C. by May.

Experiments were commenced on the 6th November, 1945. Each series was started with five gorged females that had been collected a fortnight previously from camels in Lydda.

In series I, two females began to lay eggs after 4 and 7 days respectively, and the other three after 22 to 26 days.

In series II, all the females remained alive for the whole winter and began to lay eggs in the spring ; one began 131 days after the beginning of the experiment. The daily minimum temperature had risen by then to between 14°C. and 17°C. and the maximum from 19°C. to 20·5°C. Other females of the same series began to lay eggs after 145, 168 and 196 days respectively.

In series III the females did not begin to lay until the beginning of the summer, about the 1st of June, or 200–210 days after the experiment had begun.

Hundreds of eggs laid by females at a temperature of 32°C. (series I) were transferred at the age of 1–3, 8–10, and 10–13 days to series II (in the corridor) and to series III (out of doors).

Control eggs, from series I, which were left in the thermostat, developed quickly and larvae hatched 14 to 22 days later.

Eggs transferred to the corridor at the age of 10–13 days developed to larvae in 20 days, and those aged 8–10 days in 34 days. Some of those aged 1–3 days shrivelled and died about the middle of January after having remained alive for about seven weeks, whilst others of the same batch developed fairly well, but shrivelled and died in the middle of February after having lived for about 12 weeks.

The eggs which were transferred to series III (out of doors) at the age of 10–13 and 8–10 days developed very slowly, and subsequently died at the beginning of

April (after 130 days). Eggs transferred to series III at the age of 1–3 days did not complete their development and died after 60 days.

Young larvae, 2–4 days old, transferred from the thermostat to the corridor, lived for about one month, whilst those transferred to the window (series III) lived for about two months. It has been observed (Feldman-Muhsam, 1947) that larvae hatching from a batch of eggs normally form a cluster in order to protect themselves against unfavourable external conditions. At a low temperature, the longevity of unfed larvae varies between wide limits. At 17·5°C. unfed larvae live on an average 14 days at a relative humidity of 20 per cent., and 163 days at a relative humidity of 95 per cent. Under the latter conditions a specimen could be kept alive for 241 days.

Ungorged nymphs which were introduced into series III on the 3rd February were still alive at the end of April, when they were used for another experiment.

Gorged nymphs, which had been kept for 4 days in the thermostat after leaving their host, were transferred to the corridor on the 25th January, 1946. They developed normally and gave five imagines after 50 days, and an additional nine after 125 days, on 1st June. Other nymphs which were transferred to series III (out of doors) only reached adult stage at the beginning of summer, on the 1st July.

Gorged larvae kept at a constant temperature of 17·5°C. and at various humidities (20–95 per cent. R.H.) metamorphosed to nymphs in from 30 to 50 days. The metamorphosis of the nymphs at this temperature seems to depend on the relative humidity. At a high relative humidity of 90–95 per cent. the nymphs lived for a very long time without changing and most of them finally died. The nymphal stage of the few individuals which ultimately developed into imagines lasted for 400–500 days. At a lower relative humidity about 40 per cent. of the nymphs produced adults after a nymphal stage lasting 114 to 348 days.

Conclusions and Summary.

Hibernating gorged females of *Hyalomma savignyi* remain alive and begin to lay viable eggs with the onset of favourable conditions in a room in the middle of March and out of doors in the second half of May.

The gorged nymphs hibernate and metamorphose to imagines by the middle of March at room temperature or the middle of June, out of doors.

The eggs seem to be the most vulnerable stage of the life-cycle and no development into larvae took place during the winter, out of doors, irrespective of the age of the eggs. At room temperature partly developed eggs continued their development but younger eggs died.

The survival of unfed larvae depends on the size of the cluster that the larvae form and on the temperature and humidity.

Unfed nymphs can endure winter conditions in sheltered places, but not as well as gorged ones.

At low temperature, metamorphosis of nymphs to imagines is unfavourably affected by high humidity, while that of larvae to nymphs does not depend on humidity conditions.

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CLIMATE AND THE ACTIVITY OF THE KENYA COASTAL *GLOSSINA*.

By J. Y. MOGGRIDGE.

Department of Tsetse Research, Tanganyika Territory.

This paper explores the effect of the climate on the activity of *Glossina pallidipes*, Aust., *G. austeni*, Newst., and *G. brevipalpis*, Newst., under Kenya coastal conditions.

A study of *G. pallidipes* in the semi-arid country about Shinyanga in the Lake Province of Tanganyika Territory has disclosed a reaction to climate very different from that depicted in this paper. Whereas in Shinyanga a very strong positive correlation has been shown to exist between the numbers of *G. pallidipes* caught and the evaporating power of the air (Potts, 1940), there is a negative correlation between catch and saturation deficit on the Kenya coast. It appears that, in Shinyanga, peak activity of *G. pallidipes* coincides with maximal dryness, while on the Kenya coast peak activity coincides with maximal humidity; and when under Kenya coastal conditions marked dry season activity is observed, this activity is confined to the early hours of the day.

In Zululand *G. pallidipes* behaves in a manner similar to that recorded for Shinyanga (Harris, 1930) but, in certain inland parts of Kenya and Uganda, the reaction of the tsetse to climate is similar to that recorded for the Kenya coast (Lewis, 1936, unpublished report).

Climate.

The climate of Kilifi is greatly influenced by the trade winds. The N.E. trade wind begins in November and lasts till February and during this period the weather becomes gradually drier and hotter. The S.E. trade wind, bringing rain, blows from the end of March to September inclusive.

It is unusual for a month to pass at Kilifi without rain. January and February are months of light rainfall but in other months rain usually falls in considerable quantities. December is a month in which there may be heavy rain forming a wet spell between two comparatively dry periods. For the purpose of this investigation three seasons have been recognised. They are as follows :—

The wet season ... April, May and June or May, June and July according as the rains come early or late.

The humid season ... June or July to October.

The dry season ... November to March (may be broken by a wet spell in December).

The curve of the mean monthly maximum temperatures throughout the year shows that the lowest mean daytime temperatures are experienced in June and July. From this level the temperature curve rises steadily until a peak is reached in January.

A fall in temperature during December may be brought about by rain and February is usually less hot than either January or March. During March the temperature may equal that of January, but in April the first rains may be expected, and the temperature curve falls steadily after that to its lowest level in June and July. There is little change in night temperatures throughout the year.

Meteorological stations were established for the period of the observations, in thicket growing on coral rag rock, in clump thicket and in forest. Each station included a thermohygrograph.

Fly Rounds.

Fly round technique has been described by Potts (1930) and, in the main, the methods described by this worker have been followed at Kilifi. Fly rounds were laid out through representative vegetation and the rounds were divided according to vegetational and other features into numbered sections. The fly rounds referred to in the text are as follows :—Forest Round, Savannah Round, Clump Thicket Round, Coral Rag Thicket Round and Elephant Thicket Round. Experience showed that it was advisable to restrict fly rounds to a maximum length of about 6 miles, because interest flagged over a greater distance under trying conditions of heat and humidity. A further consideration was that if the morning rounds were carried on beyond the time taken to complete 6 miles it was found that tsetse activity had fallen to such a degree that few more were caught. Preliminary reconnaissances showed that, although the catching screen (Swynnerton, 1936) attracted more tsetses to the party than men by themselves, it nevertheless failed to attract them in sufficient numbers. This was particularly the case in regard to *G. austeni* and *G. brevipalpis*. On all fly rounds, therefore, two trained bait cattle were used.

The party on fly rounds usually consisted of seven Africans. The African in charge carried notebook and pencil, a watch and whirling psychrometer. Two fly-catching Africans, each equipped with a fly net, accompanied each bait animal. The paint boy carried a tin containing the paints for the day and a small pointed stick for applying them to the tsetses. A herdsman always walked in front of the first animal which was trained to follow him. Each tsetse as it was taken from the net was handed to the head boy for determination of sex, species, and hunger stage (Jackson, 1933) in the case of *G. pallidipes*, and of *G. austeni* on the forest round only ; but a record was made of all *G. austeni* containing blood visible from external examination. After painting, the tsetses were released. At the start of each section the time was recorded as well as the amount of sun, wind and cloud. Readings of the whirling psychrometer were taken at a halt made near the middle of each section. The rounds were made in the morning starting at 7 and, in addition, some from 3.30 in the afternoon, each on average, six times a month. The round from No. 1 section to the end section on one day was reversed on the next day but one.

Results obtained from fly-round Data.

1. *Activity.*

The statistical treatment to which the data have been subjected has been based on the assumption that fluctuations in density-activity figures from fly rounds *within* any one month are caused by changes in activity rather than in true density. Individual catches were expressed as deviations, positive or negative, from the monthly means. These deviations were found to be large when the mean catch happened to be high, probably because true density was high. The deviations were, therefore, corrected by dividing by the monthly means and multiplying by 100 ; the results then represented percentage rise or fall in activity about the monthly mean. The saturation deficit (s.d.) at the time of catch (taken usually from readings of the whirling psychrometer) was also expressed as deviations above or below its monthly mean.

(*a*) *G. pallidipes* in coral rag thicket which consists of continuous evergreen thicket growing on shallow soil overlying " coral rag " rock.

No correlation was found between activity and s.d. on morning rounds. This is thought to be on account of the small range in s.d. readings.

On afternoon catches activity and s.d. at time of catch showed significant negative correlation over 19 observations, $r = -0.45$ and P between 0.05 and 0.02. A stronger correlation was found between tsetse activity and temperature on afternoon

catches. It has been found that dry bulb temperatures at 4 p.m., taken from the thicket thermohygrograph, are close to the mean dry bulb temperature for the day during the hours 8 a.m. to 6 p.m. A strong negative correlation existed between tsetse activity in the afternoon and the 4 o'clock reading of the dry bulb on the afternoon of the catch, $r = -0.7695$ and P less than 0.01.

(*b*) *G. pallidipes* in clump thicket consisting of separate massive thickets isolated by narrow grassy glades.

Morning catches only were made on this round. Rounds started on November 9th, 1936, and were discontinued on October 28th, 1937, the average being rather more than 6 rounds each month.

A significant negative correlation was found to exist between tsetse activity and s.d. at time of catch over 66 observations, $r = -0.4752$, P less than 0.01. Temperature at time of catch was shown to have a significant negative correlation with tsetse activity ($r = -0.3806$), but there was no significant difference between this and the correlation of activity with s.d.

(*c*) *G. pallidipes* in elephant thicket—tall, robust evergreen thicket on coral rag.

Morning catches only were made, the rounds starting on February 5th, 1937 and continuing until February 25th, 1938. On the average 5 rounds were made each month.

On section 1 (regenerating thicket five years old) numbers were high but higher catches were made when the round started from that section, and consequently earlier in the morning, than when the round was made in the reverse direction and section 1 traversed later in the morning. The data from this section have been examined in order to ascertain whether the degree of generally lower activity later in the morning was related to the degree of difference in the s.d. ; whether increased activity was caused by reduced s.d.

The late morning catch was expressed as a percentage of the early morning catch for each pair of days. The early morning s.d. was similarly expressed as a percentage of the late morning s.d. The deviations from the respective means of both sets of percentages were taken and the correlation worked out. The result showed that the degree of generally lower activity on section 1 over 20 pairs of observations was significantly related to the degree of difference in the s.d., $r = -0.8330$ and P is less than 0.01. A large part of the contribution to this correlation is due to a pair of days in October when the late morning catch was higher than the early morning catch and the early morning s.d. was higher than the late morning s.d. However, if this pair of days is disregarded, the correlation is $+0.5967$ and P is still less than 0.01.

The data were examined statistically in order to ascertain whether temperature at the time of the catch was significantly correlated with the difference in tsetse activity in section 1. The method used was similar to that just described but temperature was substituted for s.d. The result showed $r = +0.3188$ and P more than 0.1. The correlation is, therefore, quite insignificant. Examination of the two correlations (using z) showed that the latter was significantly lower than the former. Therefore s.d. affected activity more strongly in this instance than temperature.

The same methods were applied to the data from a section which passed along a motor road and on which tsetse activity was very high. Quite insignificant correlations existed with both s.d. and temperature. It is assumed that it was the conditions within the thicket that influenced the activity of tsetses found on the road, and not the conditions obtaining on the road.

The data from section 1 were examined in order to find out whether the fall in activity that took place between early morning (7 a.m.) and late morning (9 to 10 a.m.) could be entirely explained by the rise in s.d. The mean catch on the early morning rounds for the year (February to November) was 53 and on the late morning

rounds 6. The mean early morning s.d. was 3·1 millibars and late morning 10·3 millibars. It was found that (judging by the other results already mentioned) the fall in activity could not be entirely explained by the rise in s.d. It is thought that the tsetses became " tired " (K. Mellanby in correspondence with Dr. Jackson) after the initial early morning burst of activity and in seeking an explanation for falling activity this factor of " tiredness " must be added to the influence of rising s.d. On the motor road section, also, tsetses were about $3\frac{1}{2}$ times as active earlier than later in the morning, and this difference in activity cannot be explained except by considering " tiredness " as a contributory factor to the influence of rising s.d.

(d) G. pallidipes in savannah (Isoberlinia-Brachystegia woodland).

This round was made both in the mornings and the afternoons from May, 1936, to the end of April, 1937, 3 times each month.

No correlation could be found between activity and s.d. on morning catches. It is thought that, as in the coral rag thicket, the range of variation between readings was too small to influence activity.
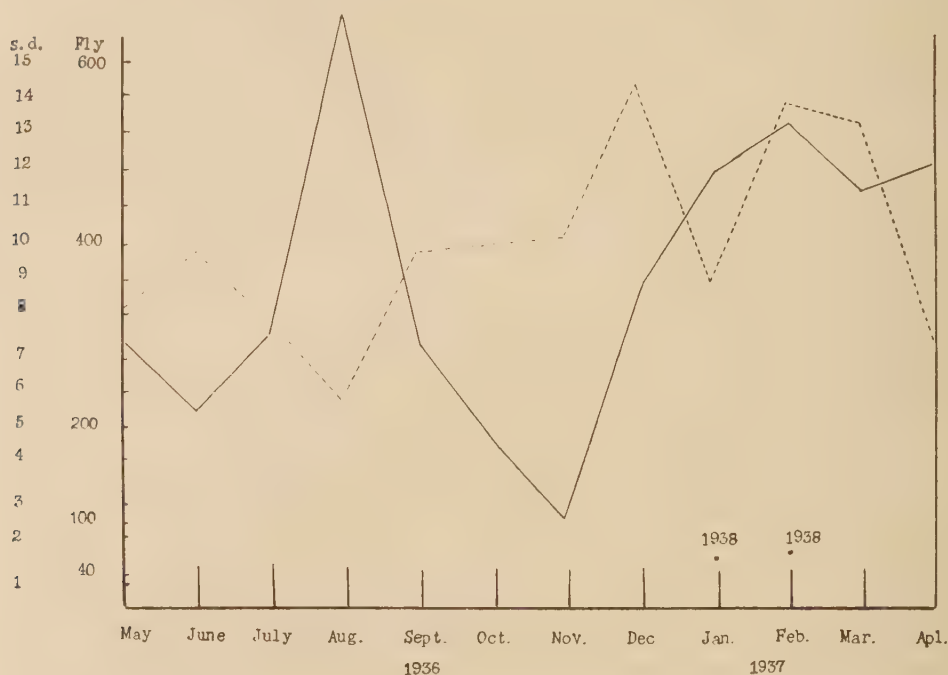
Fig. 1.—Savannah Round. Total morning catches of G. pallidipes shown ——————.
Afternoon saturation deficit from whirling psychrometer shown ----------.
Note.—Catches made during the mild dry season of 1938 are shown for comparison.

A significant negative correlation, however, existed between mean monthly morning activity and mean monthly afternoon s.d. taken from afternoon rounds for the period May to November 1936 (fig. 1). There were no recording instruments in the savannah and so it is not possible to examine the relationship between morning activity and the afternoon s.d. of the day before. A negative correlation existed between these two factors, r= —0·6068, P between 0·02 and 0·01 with 16 observations over the period May to November. Afternoon data showed that in comparison with

morning catches and with afternoon catches on other rounds, tsetse activity in the savannah was very low throughout the year.   This is accounted for by the influence of s.d. at the time of the catch.   It would seem, then, that the suppression of activity in the afternoon by high s.d. raised the figures on the morning catches.   Thus it would appear that morning activity in the savannah was strongly influenced by the s.d. of the previous afternoon, but it is also possible that temperature and not s.d. may have been the operative factor.   This is suggested from results obtained on other rounds, but the relationship between the two factors is so close that it is possible that either or both may have been responsible.

During the dry season from December to March, the negative correlation between morning activity and afternoon s.d. ceased to exist.   During that period on the contrary, a significant positive correlation appeared between morning activity and afternoon s.d., $r = + 0 \cdot 6357$ and P less than $0 \cdot 01$, and there were no negative values in 17 observations.   Thus, during the humid season of the year from May to November, a significant negative correlation existed between morning activity and afternoon s.d., while during the dry season from December to March a strongly positive correlation set in between the same two sets of factors.   This positive correlation was carried on into the humid month of April, when a lowering of the high s.d. brought about a collapse of the dry season activity.   This will be discussed later under " rush activity ".

(*e*) *G. pallidipes* in Sokoke Forest (coastal).

Morning rounds were made from March 8th, 1937, to February 18th, 1938, rather more than five times each month, and afternoon rounds from July 5th, 1937, to February 24th, 1938, four times each month on an average.

A significant negative correlation existed over the whole round between morning catches and s.d., $r = -0 \cdot 30$, P between $0 \cdot 05$ and $0 \cdot 02$, with 50 pairs of observations.

On section 5, where the instruments were located, which passed through a long stretch of homogeneous forest, a significant negative correlation existed between morning activity and s.d., $r = -0 \cdot 44$, P less than $0 \cdot 01$ in 44 observations.   The tsetse data from morning catches on section 5 were examined in relation to temperature at the time of the catch.   These gave a significant but lower negative correlation, $r = -0 \cdot 335$, so that s.d. gave an insignificantly better correlation with activity than temperature.

On section 5 for afternoon catches during the period July to December, a significant negative correlation existed with 4 p.m. temperatures (taken from a thermohygrograph in section 5) on the day of the catch, $r = -0 \cdot 4260$, P between $0 \cdot 05$ and $0 \cdot 02$, with 22 observations.

On the above section, the afternoon activity was also tested with s.d. at time of catch.   The result showed that the correlation between the two factors was not quite significant, $r = -0 \cdot 4085$; the significant point in the Table of r is $0 \cdot 4227$.

(*f*) General observations on activity of *G. pallidipes*.

An examination of the data suggested that activity might vary with percentage change rather than with actual change in s.d.   This possibility was tested by correcting the s.d. deviations from the monthly means, of which they were shown as a percentage, in the manner already explained for the tsetse figures.   This treatment was applied to the clump thicket round and to section 5 of the Sokoke forest round as being the most suitable data available.   The strength of the correlation was in each case slightly lower than before.   For the clump thicket $r = -0 \cdot 3566$ and on section 5 of the Sokoke forest round $r = -0 \cdot 4191$, whereas previously the respective values were $-0 \cdot 4752$ and $-0 \cdot 4351$.   By the new method P is still less than $0 \cdot 01$ in both cases, but clearly the first method is to be preferred.   In other words, activity is apparently determined by actual changes in s.d. rather than by percentage changes.

An attempt was made to find the relation (regression line) between actual change (rise and fall) in s.d. and percentage change in tsetse activity. The percentage deviations from the monthly means of density-activity were taken for clump thicket and section 5 of the Sokoke forest round and coral rag round evening catches, and actual deviations from monthly means of s.d. For each value of s.d., without regard to its sign, were written down corresponding values of corrected tsetse figures from the combined rounds, a negative sign being given to the tsetse figure when its sign was the same as that of the corresponding s.d. A positive correlation could, therefore, be expected. Grouping the millibars 0 to 1, 1·1 to 2, etc., the following mean percentage change in tsetse is obtained :—9, 11, 30, 42, 40, beyond which there are too few readings. A line drawn through these points shows that roughly for a change of 1 millibar in s.d. there will be a change of about 10 per cent. in tsetse activity.

(g) *G. austeni* in coral rag thicket.

G. austeni was always captured in greater numbers in the western than the eastern half of the thicket, and so the data from the western sections 14 to 21 have been chosen for examination. The methods used in statistical examination of the data were the same as those applied to *G. pallidipes*. Morning activity showed negative correlation with s.d. at the time of the catch, r= —0·5694, P less than 0·01 with 63 observations. A change of 1 millibar in s.d. is estimated to bring about a change of about 12 per cent. in activity.

G. austeni was evidently more responsive than *G. pallidipes* to the small changes in s.d. that occurred in the mornings in the coral rag.

(h) *G. austeni* in clump thicket.

Activity and s.d. at time of catch showed a negative correlation, r= —0·2575, P between 0·05 and 0·02 with 64 observations.

From monthly means, density-activity for both males and females showed a *positive* correlation with mean monthly s.d. (see fig. 2).
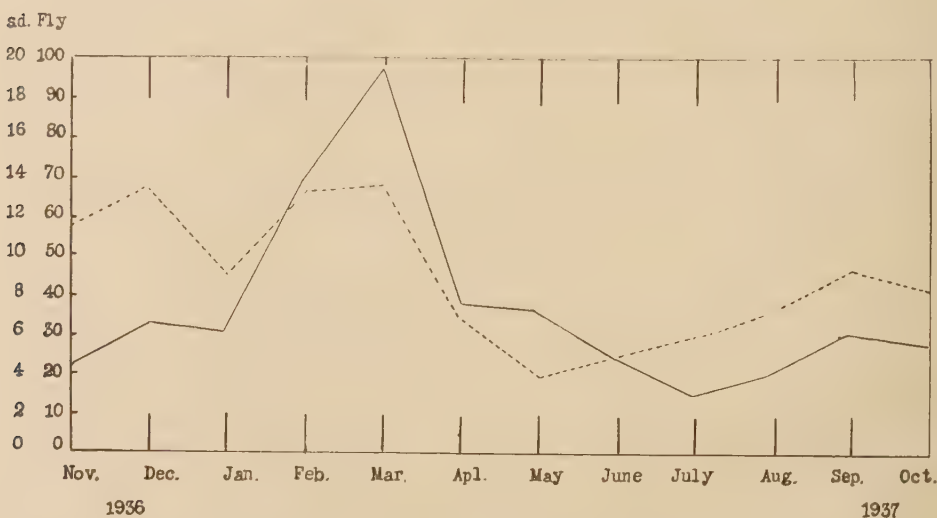
Fig. 2.—Clump Thicket Round. Morning catches of *G. austeni* shown ————.
S.D. at time of catch shown ----------

For males r= +0·68 and for females +0·65. P is about 0·02 for both sexes. This positive correlation between mean monthly density-activity and mean monthly s.d. is interesting and is in marked contrast to the findings of the reaction of this tsetse and *G. pallidipes* in other vegetation types. It has been stated above that a negative correlation existed between activity and s.d. at time of catch. In order to arrive at an understanding of this apparent paradox, it is necessary to remember that the fly round was not made through thickets but, for the most part, in open glades between thickets. In June and July, during the wet season, mean monthly density-activity showed a negative reaction to monthly s.d. This seems to suggest that for only two months in the year did humidity outside the thickets equal that inside. The effect of high s.d. outside the thickets was to prevent the passage of tsetse across the open either in normal flight or with intent to attack an object in the open. At all times humidity was high in the early mornings and consequently as the mean monthly s.d. rose, and activity across the open became restricted, more and more tsetse were compelled to make their flights into the open during the early hours of the day. As the s.d. fell and the period of activity in the open became less restricted fewer tsetses were taken on rounds in the early mornings. Finally, during June and July, humidity inside and outside the thicket were the same throughout the greater part of the day, resulting in small catches being made during the comparatively short time taken to make the fly round. During these two months *G. austeni* showed a negative correlation between mean monthly density-activity and mean monthly s.d. as on other rounds.

(*i*) *G. austeni* in the Sokoke Forest.

There existed on section 5 of the Sokoke forest round a strong negative correlation between activity and s.d. at the time of the catch. r= −0·4930, P less than 0·01, with 51 pairs of observations.

Once again it appears that *G. austeni* is sensitive to changes in s.d. even when the s.d. is quite low, conditions under which *G. pallidipes* fails to respond.

(*j*) *G. brevipalpis* in coral rag thicket.

Only one correlation has been worked out for this tsetse in connection with s.d. Afternoon catches in the coral rag thicket from May to December, 1936, treated as mean monthly density-activity figures, showed a significant negative correlation with the mean monthly s.d. during daylight hours calculated from thermohygrograph readings.

2. *True Density of* G. pallidipes.

" True densities " were calculated from apparent density (density-activity) by correcting the apparent density to what it should have been at s.d. of 10 millibars on the assumption that 1 millibar change in s.d. causes a 10 per cent. change in activity (see above).

Two examples are given which show the method used.

Example : Coral rag, May. The mean morning catch of both sexes for this month was 137 and s.d. 11·5 mb.=1·5 above 10. Expected activity should, therefore, be 15 per cent. less than the unit activity at 10 mb. Therefore, true density index

$$=\frac{13700}{100-15}=161.$$

Example : Coral rag, June. Tsetse 332 and s.d. 4·2 mb. or 5·8 less than 10 mb. Expected activity is, therefore, 58 per cent. above unit activity at 10 mb. True density index$=\dfrac{33200}{100+58}=210.$

Obviously, however, if the s.d. is 20 the apparent density on the above formula will be corrected to infinity because the denominator will be 100−100=0. Clearly, the conversion formula will only work at moderate or low values of s.d. Although

the regression line of activity cannot be straight, it seems that at moderate s.d. it can be taken as such.

"True density" here is taken to mean the remaining density-activity complex after the subtraction of the very considerable proportion of activity which appears ascribable to the effect of saturation deficit as measured at Kilifi. It must, therefore, be looked upon as an approximation only, with a relatively small amount of activity included in it.

Correlation between true density and s.d. from thermohygrograph readings could be tested from coral rag and clump thicket data only, as these were the only two rounds to have recording instruments placed in them for a time long enough to supply sufficient data. The results showed that significant negative correlations existed between calculated true density and s.d. when both were treated as monthly means. It is apparent, therefore, that true density varied inversely as s.d. (at least on these two rounds).

It was said above that no correlation existed between morning catches of *G. pallidipes* in the coral rag thicket and the s.d. at the time of the catch. This argues that these catches were little affected by s.d. which seemed the most important factor influencing activity. Therefore, it seems legitimate to suppose that the mean monthly variations in the coral rag catches represented mainly true density. The curve of "true density" does in fact follow very closely that for the mean monthly density-activity figures on the coral rag round. This is true to some degree also of the fluctuations on the other rounds, and so it may be said that the tsetse population reaches maximum density during the period of maximum humidity in the wet season. As the dry season sets in, the density of the population decreases until it reaches a very low level indeed during the period of December to March. In April the conditions moderate with the first rains and from that month density increases until a peak is reached in May or June in normal years. Nash (1937) concludes that "excessive evaporation, high maximum temperature and a great diurnal temperature range are inimical to high fly density" and states that "it was found for both species (*G. morsitans* and *G. tachinoides*) that as maximum temperature rises so longevity decreases, and that as the maximum temperature falls so longevity increases". Nash is of the opinion that his experience in West Africa would not cause him to modify his conclusions in respect of East African tsetses.

3.   *Rush Activity.*

     (a) *G. pallidipes.*

A violent positive reaction of *G. pallidipes* to the severe dry season conditions of the first three months of 1937 was first seen in the savannah (fig. 1) and, although the evidence of sudden very marked activity could not at once be accounted for, it was probably caused by concentration of activity within the limited hours of tolerable conditions. This concept led to the term "rush activity". Day catches of *G. pallidipes* during the periods of rush activity gave absolutely no evidence that the mean hunger stage of the tsetses had increased ; also the few specimens of *G. pallidipes* that were taken on special night catches showed no evidence of hunger and it must be assumed that the period available for activity was sufficient for them to make contact with their hosts. There is reason to think that under canopy the period available for rush activity would be from 6 a.m. to 10 a.m.

It is thought that the dry season upward curve and peak, which coincided with the very severe conditions of the early months of 1937, was brought about in the following way. Under severe dry season conditions, temperature and s.d. rise very rapidly after an initial early morning period of comparatively low temperature and high humidity. The effect of high temperature and high s.d. throughout the day is to render the tsetses inactive and as the season progresses the period available for activity is proportionally reduced. Temperatures are low in the mornings and

humidity is always very high and, into the few hours available to it under these conditions, the tsetse population concentrates the activity that it would under normal conditions spread over the greater part of the day. At such seasons, the catch of *G. pallidipes* varied from about 200 to 400 per hour at 7 or 8 a.m., with 35 to 50 per cent. females in the catch.
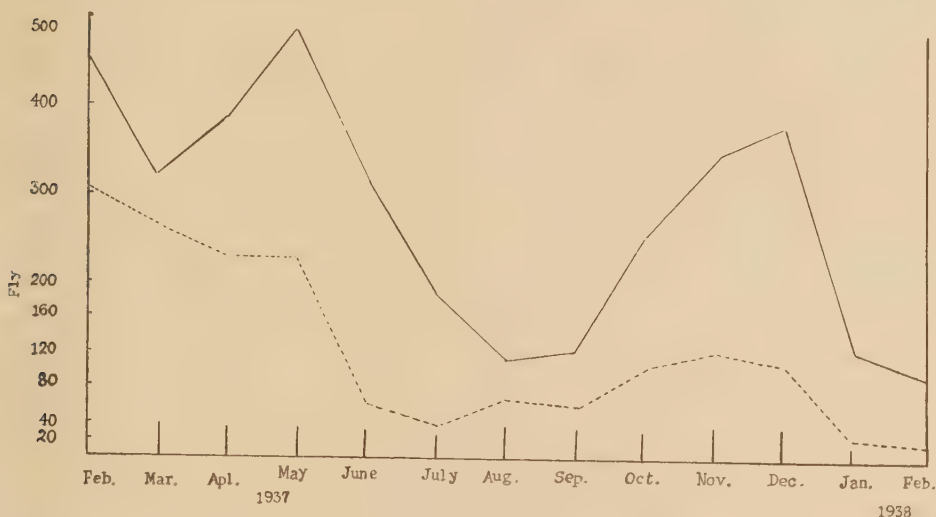
Fig. 3.—Elephant Thicket Round. *G. pallidipes.*
Section Nos. 1 and 10 shown—— — —.
Section Nos. 2, 4 to 8 shown ----------

The theory that rush activity is brought about by restricted activity induced by the excessively high temperatures and s.d. of the previous day receives confirmation from the reaction of *G. pallidipes* in the elephant thicket to the mild dry season conditions of 1937–38. It is reasonable to suppose in connection with the theory of rush activity that tsetses living under conditions of deep shade will feel the influence of mounting temperatures and s.d. less than those living under conditions more exposed to the influence of these two factors. The vegetation on section 1 of the elephant thicket round was composed of fine stemmed deciduous recent regeneration, and the vegetation within the elephant thicket proper was much heavier. Figure 3 shows the mean monthly density-activity figures for section No. 1 and for the sections passing under normal thicket canopy, which made up the remainder of the round, with the exception of the marginal section No. 3 and the Malindi road section. It will be seen from the figures for February, 1937, that, under the severe conditions of the dry season of 1936–37, the tsetses in both kinds of thicket showed marked rush activity. In October, November and December, 1937, during the milder dry season of 1937–38, tsetses within the thicket proper under typical thicket canopy showed little indication of this. On section No. 1, however, tsetses within the recent regeneration showed rush activity on a scale similar to that displayed during the previous severe dry season. Owing to conditions becoming less severe on that section during January and February, 1937, all indications of rush activity disappeared and the figures for February, 1937, and February, 1938, are in interesting contrast.

(b) *G. austeni.*

This tsetse showed very marked rush activity in all vegetational communities during the severe physical conditions which marked the first months of 1937. On

the Sokoke forest round only one month (March) is recorded because the round was started then. In that month an average catch of 168 *G. austeni* was recorded. The average catch over the remaining 11 months during which the round was made averaged 74.

On the clump thicket round *G. austeni* showed marked rush activity and the peak was reached during March, 1937 (fig. 2).
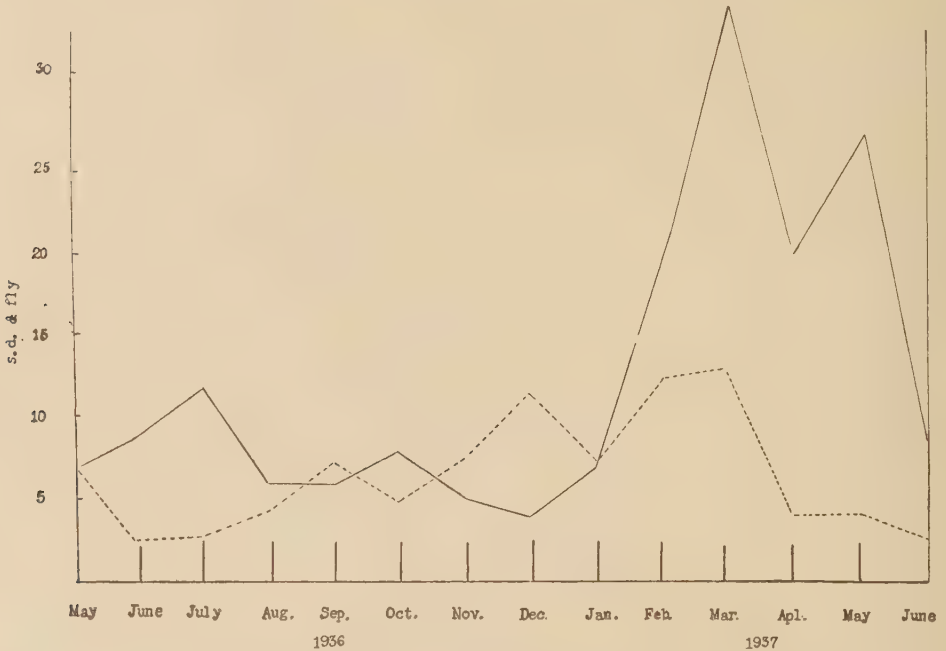
Fig. 4.—Coral Rag Thicket Round.
Morning catches of *G. austeni* (sections 14–21) shown ——————
S. D. at time of catch from whirling psychrometer shown ---------

In contrast to *G. pallidipes*, *G. austeni* in the coral rag thicket reacted violently to the severe physical conditions. The degree of activity displayed is shown in figs. 4 and 5.

Activity all-day Catches.

In order to obtain a picture of the hour to hour activity displayed by *G. pallidipes* and *G. austeni* throughout the day, a series of all-day catches was made in the savannah and the coral rag thicket. Catches started at dawn and continued until close on sunset. They were made for ten minutes every hour along selected stretches of the two rounds. Two bait oxen and four catchers were used and the writer was present at all catches to record the data. Readings of the whirling psychrometer were taken during the catches. Tsetses were hunger staged, sexed and then released. During periods between catches, the party rested some distance from the catching area. At the start of each 10-minute catch, all tsetses captured were killed. This was done to ensure that those attracted by the movement of the party from the resting place should not be included in the catch. During the catch the party moved slowly along for 200 yards and then retraced their steps.
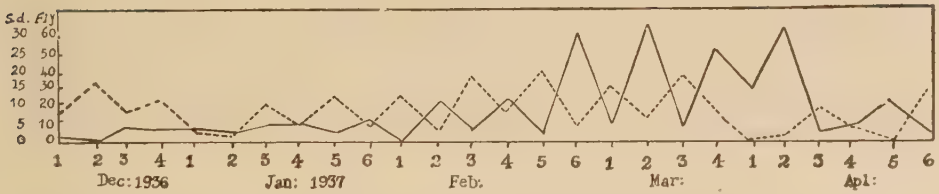
Fig. 5.—Coral Rag Thicket Round. Individual catches on sections Nos. 14–21.
Morning catches of *G. austeni* shown ————.
S.D. at time of catch from whirling psychrometer shown ----------

G. pallidipes.

During March and April of the dry season of 1939, nine all-day catches were made in section 2 of the savannah round.

The data obtained from these catches are set out in fig. 6 together with the data obtained in ten all-day catches during the wet season, in May and June, 1939, over the same section of the savannah round.
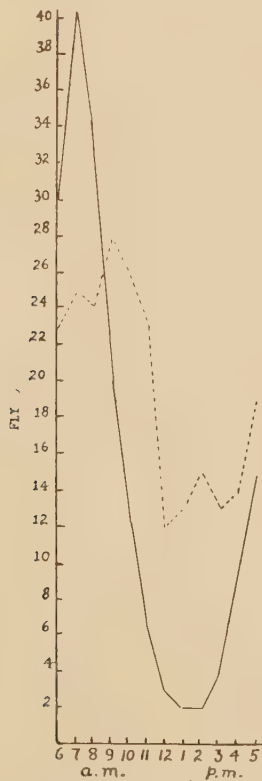
Fig. 6.—Average number of *G. pallidipes* taken for ten minutes hourly from dawn to dusk during the dry and wet seasons 1939 in Savannah wooding.
Dry season, nine catches, shown ————.
Wet season, ten catches, shown ----------.

It will be seen that, although generally similar activity is displayed at corresponding hours during the dry and wet seasons, the wet season catch is very nearly $2\frac{1}{2}$ times as large as that of the dry season. Activity in both seasons starts on a large scale soon after light and increases to maximum proportions for an hour after daybreak. From that time activity diminishes throughout the day until late afternoon when increasing activity is shown until sunset.

G. austeni.

The hour to hour daytime activity of *G. austeni* was studied in a section of the coral rag thicket, a short way north of the coral rag thicket round. The procedure was the same as that described above for all-day catches in the savannah. During the dry season, December 1937 to April 1938, 18 all-day catches were made and during the wet season, May 1938, four all-day catches.

The hourly activity curves for the dry and wet seasons are very similar. Maximum activity was recorded at 6 a.m. and from then until 10 a.m. activity decreased remaining at a very low level throughout the day. In the dry season only a very slight degree of activity was shown at 8 p.m. The average daily catch during the dry season was 49 and that for the wet season was 40.

Constitution of the Catch.

G. pallidipes.

Data on teneral (freshly emerged) tsetses were kept for *G. pallidipes* only. On occasions when there appeared to be a large proportion in a catch of *G. austeni* a note was made of the observation.

The numbers of teneral tsetses captured on fly rounds were very small indeed, the largest monthly average recorded being 11 in the coral rag thicket. They were taken on all rounds and the numbers for individual rounds did not greatly differ. The largest numbers were taken during the wet season months of May and June, but in the savannah and the elephant thicket a few were taken during the dry season also. The average percentage of females among teneral tsetses over all rounds was 70. The distribution of teneral flies was even and it cannot be said that any preference was shown for marginal sections.

Female tsetses were at all times numerous and formed, on average, 41 per cent. of the total catches of non-teneral flies. Female activity and density appeared to be influenced by the same factors as influenced the meals of the population and in the same way. There was very little seasonal change in the female percentage of the catches.

G. austeni.

The only round from which sufficient data are available is the Sokoke forest round. On morning rounds a mean female percentage of 32 was recorded in the humid season against a percentage of 24 in the dry season. On afternoon rounds this humid season predominance was reversed, for during the dry season the female percentage was 36 and that for the humid season 31. There are indications that on this round female *G. austeni* showed a preference for marginal sections, but the preference was not very marked and on the whole the distribution of females was very even.

G. brevipalpis.

Data from the Sokoke forest round only need be considered in connection with the female activity and distribution of this tsetse. Females formed a very low proportion of the catches in both morning and afternoon. On morning catches the humid season female percentage was 4·9 and that of the dry season 5·2. On afternoon catches the humid season female percentage was seven while that of the dry season

was eleven. Females did not usually appear to the catchers until dusk when it had become almost too dark to see. At dusk males and females were taken in comparatively large numbers in copulation on the bait cattle. In daylight this was never observed. It is,perhaps, reasonable to infer from this evidence that the females of *G. brevipalpis* were at all times present in the vicinity of the party when the males were being taken, but that they did not attack until dusk.

Recaptures.

During the course of the investigation (up to the end of January, 1938), 71,913 tsetses of all three species were marked with oil paints to show date and place of original capture. They were released immediately after painting and, judging by the vigour with which all but young tsetses flew away, they suffered no harm when being held for the application of the paint. Table I gives the salient figures in connection with the recaptures.

TABLE I.

Data relating to recaptures of all three species.

Species		Marked	Recaptured	Recaptured on same round	Average days elapsing before recapture
G. pallidipes	...	54,862	92	71 per cent.	15
G. austeni	...	12,845	6	83 per cent.	35
G. brevipalpis	...	4,206	23	87 per cent.	9

The maximum period elapsing before a marked tsetse was recaptured was that of a *G. pallidipes* (sex not recorded) which was marked in the clump thicket area on 12th February, 1937, and recaptured in the same area on 3rd July, 1937, a period of approximately five months.

Unfortunately the data in connection with the sexes are not complete in respect of recaptures of *G. pallidipes* but of the total number of *G. austeni* recaptured 80 per cent. were males, and males accounted for 91 per cent. of the recaptures of *G. brevipalpis*.

The influence of the seasons may be observed in an analysis of the recaptures of *G. pallidipes* made month by month. During the wet season from June to August, 1937, the number of tsetses recaptured was higher than at any other time.

The comparatively high numbers of *G. pallidipes* captured during the humid season may, perhaps, be taken as an indication of longevity during that season. Density-activity figures were high on all rounds, except the coral rag thicket and clump thicket rounds, during a period of rush activity in the dry season of 1936–37, but, with the exception of February, the number of recaptures during the dry season months was small. This is possibly due to the greatly curtailed length of life under dry season conditions.

Summary.

This paper deals with *Glossina pallidipes, G. austeni* and *G. brevipalpis* as they occur on the Kenya coast.

Temperature and saturation deficiency both exert a generally inverse effect on the activity and apparent density of both *G. pallidipes* and *G. austeni*. True density also varies inversely as the s.d.

" Rush activity " is a term used to express the exceptional activity displayed by both *G. pallidipes* and *G. austeni* in the morning during periods of severe dry weather. This extreme form of activity is displayed by both sexes. The theory that rush activity is really compressed activity induced by very high temperatures and s.d. later in the day is supported in a discussion of the reaction of *G. pallidipes* in two adjacent vegetation types under normal and abnormal dry season conditions.

Teneral tsetses were taken in remarkably small numbers on all rounds. Data were kept for *G. pallidipes* only. Females formed a large proportion of the catches of teneral tsetses. Teneral tsetses show no apparent preference for marginal areas ; they are more numerous in the wet season.

The activity and density of female *G. pallidipes* appeared to be influenced by the same factors and in the same way as the males. Adequate data on *G. austeni* were obtained from the forest only, but there are indications of seasonal fluctuations in female percentage. Females of both species are distributed evenly throughout the vegetation types and no marked vegetational preferences are shown. The females of *G. brevipalpis* formed only a very small proportion of the total catches.

The numbers of marked tsetses of all three species recaptured were very small indeed. The majority were recaptured after the lapse of a few days in the same locality as that in which the original markings had been made. *G. pallidipes* was recaptured in greater numbers during the humid season.

Acknowledgements.

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APPENDIX.

Monthly readings of Stevenson Screen and other standard instruments under canopy of thicket growing on coral rag rock.

Month ...	1	2	3	4	5	6	7	8	9	10	11	12
Rainfall, mm.												
1936 ...				127	102	191	70	20	35	24	24	30
1937 ...	4	0	37	121	150	75	4					
Mean maximum temperature.												
1936 ...				30·6	28·6	26·9	26·9	27·2	28·2	29·7	31·7	32·5
1937 ...	30·9	32·2	31·7	30·0	28·3	26·9						
Mean minimum temperature.												
1936 ...				22·8	22·2	21·1	21·9	21·4	20·6	21·9	21·9	22·6
1937 ...	21·6	22·8	23·3	25·6	22·5	22·2						
Solar radiation *in vacuo.*												
1936 ...				43	47	42	41	38	46	57	61	64
1937 ...	59	57	55	43	39	38						
Surface soil temperature.												
1936 ...				26·1	24·7	23·3	22·2	22·8	23·9	27·2	30·0	29·2
1937 ...	26·4	28·3	27·8	25·6	23·9	23·9						
Relative humidity per cent. at 8.15 a.m.												
1936 ...				85	90	92	90	88	87	80	75	79
1937 ...	90	87	87	91	95	91						
Saturation deficit in mb. over 24 hours.												
1936 ...					5·9	3·9	4·5	4·8	5·2	6·1	8·1	9·8
1937 ...	5·9	8·6	8·3	3·7	2·7							

Saturation deficit in mb. from whirling psychrometer, from average of morning rounds, 6.30 to 11 a.m.

Month ...	1	2	3	4	5	6	7	8	9	10	11	12
1936 ...					7·5	4·4	5·2	7·3	7·6	6·0	9·1	11·7
1937 ...	7·6	11·9	13·4	5·2	5·3							

FURTHER STUDIES ON THE LOSS OF INSECTICIDES BY ABSORPTION INTO MUD AND VEGETATION.

By A. B. HADAWAY and F. BARLOW.

Colonial Insecticide Research, Uganda.

Observations in the field (Gahan & others, 1945 ; Symes & Hadaway, 1947) have shown that mosquitos become affected after making contact with DDT-treated surfaces in rooms and may make their escape through open doors and windows. Kennedy (1947) has shown in the laboratory that sub-lethal doses of DDT excite them and that when activated they move preferentially to light. Success in the residual spraying of houses for the reduction of malaria depends upon applying a surface deposit that is lethal to mosquitos after only a brief contact.

In East Africa the majority of native houses are constructed of mud walls and grass thatch roofs. A recent survey for a field experiment in Uganda showed that 76 per cent. of a total of 3,089 houses were of this type, and that 80 per cent. had mud walls. In preliminary laboratory tests (Barlow & Hadaway, 1947), it was found that when DDT in oil solution was applied to dried mud it was deeply absorbed and only a very small proportion was present at the surface.

Field trials have been conducted in Uganda to study the effect on the tsetse population of spraying vegetation with DDT and benzene hexachloride by hand. It has been shown that there is considerable loss of insecticide by absorption into leaves when applied as a simple oil solution (Barlow & Hadaway, 1947). In some cases the initial loss was as much as 50 per cent. of the total insecticide recovered. Then followed a gradual disappearance of insecticide both from the outside and from the inside of the treated leaf.

More detailed tests were therefore undertaken to determine the efficacy of various insecticidal formulations when applied to an absorptive dried mud surface and to leaves.

Applications to Mud Blocks.

Methods.

Standardisation of Blocks.

Sun-dried soil was passed through a 40-mesh sieve and after mixing with water was made into mud blocks in wooden frames 5 cms. square and 2·5 cms. deep. The surfaces to be treated were levelled, and the blocks allowed to dry slowly at room temperature to prevent cracking.

Random samples were submitted to a series of tests, after analysis of the surface and subsurface deposits, in order to check the uniformity of the blocks used for spraying. They were found to give the same results as whole, untreated blocks although they were reduced in size by the scraping for this analysis. The results of these measurements are given in Table I.

The area of the sprayed face and the depth were measured to the nearest 0·1 cm. The depths of both scraped and unscraped blocks were determined in order to estimate the thickness of the outer layer removed, as described later. The porosity represents the percentage of the volume of the block which is air and was determined by the Standard Test for Porosity in Refractory Materials A.S.T.M. 1920 described in the Chemists' Yearbook 1940, p. 688. Three samples

of mud taken from the wall of a native hut had a mean porosity of 48·8 per cent. This higher value is undoubtedly due to the lighter packing of mud in walls. The blocks were weighed before and after drying for two hours at 110°C. and the difference expressed as a percentage of the wet weight. The loss in weight due to heating for two hours at about 500°C. was considered, after subtracting the water content, to be due to decomposition of the organic part of the mud. This further loss in weight was again expressed as a percentage of the wet weight.

TABLE I.

Sizes and properties of mud blocks.

	Dimensions			Porosity	Water Content	Organic Material
	Area	Depth				
		(a)*	(b)*			
No. of samples...	27	11	16	8	17	17
Mean and standard deviation	22·8±0·1 sq. cms.	2·4±0·0 cms.	2·1±0·2 cms.	42·4±0·6%	2·5±0·1%	12·7±0·1%
Range	21·6—24·0 sq. cms.	2·3—2·4 cms.	2·0—2·2 cms.	41·3—43·2%	2·3—2·7%	12·5—13·0%

(a)* Unscraped blocks.   (b)* Scraped blocks.

Application of Insecticide.

Unfortunately no spraying apparatus was available to produce a known and constant dosage. The required dosage was obtained by spraying a measured area of ground with a known volume of insecticide. All applications were made at a pressure of 25 lb. per square inch with a Four Oaks " Kent " Sprayer fitted with a single nozzle having an aperture of $\frac{1}{16}$-inch diameter. The mud blocks were arranged in sets of four at random over the area to be treated and each set fitted into a wooden box so that only the top faces were sprayed and insecticide was prevented from reaching the sides of the blocks directly. Dosages obtained by this crude method varied considerably.

Biological Tests.

The test insects were *Glossina palpalis*, R.-D., and *Aëdes aegypti*, L., the former being chosen because of its availability, ease of handling, and susceptibility to small dosages of DDT.

G. palpalis were bred in the laboratory from pupae collected on the shore of Lake Victoria. The flies were kept in Bruce boxes as they emerged and were given an opportunity to feed daily on sheep. Fed flies, 6–8 days old, were used in the tests and were exposed to the treated surface individually in 3×1-inch tubes which were held so that they did not touch the treated surface. Flies sat readily on the mud surface rather than on the curved walls of the glass tubes. Twenty flies were used for each exposure and, after the given time of contact, a wire gauze cap was fitted to the top of each tube which was then kept in a rack over water in a tray covered with black cloth. Mortality counts were made 24 hours after contact. The sexes of the flies used are not given as it has been found that there is no significant difference between the resistance of fed females and fed males. The mortality amongst control flies was negligible.

Specimens of *A. aegypti* were obtained from a culture kept in the laboratory at average daily temperatures of 78–80°F. maximum, and 70–72°F. minimum. A high humidity was maintained by wet blankets covering the cages. Females from

two to four days old that had been fed on guineapigs were exposed to treated surfaces individually in 2×1-inch tubes. Exposures of an hour's duration were adopted and the tubes were rested on the treated surface. *A. aegypti* respond to this method of contact with a treated surface by walking over the surface and rarely flying to the top of the tube; the few specimens that do settle on the tube are easily returned to the surface by a light tapping of the tube. The fact that the tubes rested on the mud surface did not interfere with results except, possibly, when the surface had been treated with a wettable powder and numbers of mosquitos were exposed at intervals. Some of the surface deposit may then have been rubbed off. For each test 24–32 mosquitos were used.

After contact, the mosquitos were transferred to cages (18 inches cube), covered with a wet blanket and provided with sugar solution. Mortality counts were made 24 hours after contact.

Chemical Tests.

The only feasible way of estimating the extent of absorption was by scraping away the sprayed surface of each block. Although such a method is obviously not capable of giving the absolute concentrations at different levels accurately, it was thought sufficient for comparative purposes and it does give consistent results for a given solution at a given dosage. Its main disadvantage, but one which could not be overcome, is that the smallest outer layer that it is possible to remove from a block contains more insecticide than is available to a resting insect by contact. The dosage found in this outer layer by chemical means and the toxicity of the block to insects were therefore not strictly comparable. However, both methods divide the spray formulations into the same broad classes, which are described below under the headings of oil solutions, emulsions and dispersible powders. Another disadvantage is that it is not possible to remove any layer completely; fine particles will always be rubbed into the layer immediately beneath. The standard procedure finally adopted was as follows.

Four mud blocks 5 cms. square, constituting a sample, were scraped and the layers extracted together. A sharp narrow-bladed scalpel was used and with this 0·25 g. of mud were removed from the outside of each block as evenly as possible. With practice this weight could be approached closely by a single scraping over the whole block and adjusted accurately to the final weight with a few light scrapes. The total outer layer from four blocks, weighing 1·00 g., was transferred to a soxhlet thimble. A heavy surgical knife was then used to remove the layer containing the remainder of the insecticide. This second layer weighed 10·00 g. from each block and the 40·00 g. thus obtained were placed in a second soxhlet thimble. Occasionally a third layer of 1·00 g. from each block was removed and extracted to make certain that all the insecticide was being recovered.

The depths of the layers chosen were the result of some preliminary experiments in which a series of shallow layers were removed until no more insecticide could be recovered by extraction. The total weights of mud thus removed from each four blocks before the last of the insecticide was recovered were as follows : DDT in diesoline, 35·0 g. ; 1 : 1 power kerosene and cotton seed oil, 31·0 g. ; DDT in power kerosene, 26·0 g.

The smallest weight that could be removed satisfactorily from a single block in an even layer is about 0·25 g., and 10·00 g. for the second layer represents a depth to which no spray solutions, at the dosages so far used, have penetrated. The third layers mentioned above have never contained insecticide. The relationship between weight and depth can be seen from the external dimensions given in Table I. The difference in depth between an unscraped and scraped block is 3 mms. ; this represents the surface and subsurface layers and therefore the depth of the outer layers is 1/41 of 3 mms., or 0·07 mms. approximately.

An attempt to remove the layers by means of a microtome was abandoned because of the difficulty of maintaining the knife edge and the block surface in the same plane.

The thimbles containing the mud samples were extracted with petroleum ether, boiling range 60–70 or 70–80°C., for two hours. After the extracts had been evaporated almost to dryness, the DDT or benzene hexachloride in the residue was determined by the dehydrohalogenation method (Neal & others, 1944). Blank determinations were made with unsprayed mud but were negligibly different from the reagent blank. Results are expressed as mgms. of DDT or benzene hexachloride per sq. ft.

Treatment of Blocks with Solution.

Solutions containing 5 per cent. DDT (pp′) were made from the commercial product (containing 81 per cent. para para isomer) with the following solvents :—

   (i) Diesoline.
   (ii) Power kerosene.
   (iii) Power kerosene and cotton-seed oil, 1 : 1.
   (iv) Cotton-seed oil.
   (v) Low boiling kerosene extract (Shell), boiling point range 106–125°C.
   (vi) High boiling kerosene extract (Shell), boiling point range 180–280°C.
  (vii) 30 per cent. Triton X-100 in power kerosene.

Results obtained from mud blocks sprayed with these solutions are given in Tables II and III. It is seen that absorption was considerable and that even where the largest proportion was recovered from the outer layer it amounted to only 19 per cent. of the total insecticide applied. Toxicities of the blocks to test insects were low ; kills of *G. palpalis* after 15 mins. contact varied from 5 to 40 per cent., and kills of *A. aegypti* after 1 hour contact were negligible.

TABLE II.

Recovery of insecticide from mud blocks treated with DDT/oil solutions.

Solvent			Total dosage mgms. DDT/sq. ft.	% in outer layer	Mean % in outer layer
(i)	...	...	149, 174, 191, 208, 256	8, 7, 12, 6, 6	8
(ii)	...	...	143, 174, 177, 210, 214	13, 13, 12, 14, 12	13
(iii)	...	...	206, 225, 239, 347, 367	11, 12, 10, 11, 9	11
(iv)	...	...	439, 447, 568, 619, 805	11, 13, 10, 8, 8	10
(v)	...	...	157, 185, 251, 315, 321	20, 22, 18, 19, 17	19
(vi)	...	...	167, 191, 247, 274, 341	16, 18, 12, 10, 11	13
(vii)	...	...	215, 216, 396, 405, 444	15, 15, 11, 9, 10	11

TABLE III.

Toxicity of mud blocks treated with DDT solutions to test insects, and recovery of insecticide from the same blocks.

Solvent			mgms. DDT/sq. ft.		% mortality of *G. palpalis* after 15 mins. contact	% mortality of *A. aegypti* after 1 hour contact
			in outer layer	Total		
(i)	...	...	15	256	5	10
(ii)	...	...	30	210	20	10
(ii)	...	...	29	208	25	15
(iii)	...	...	23	206	25	10
(iv)	...	...	52	619	15	10
(v)	...	...	59	315	40	—
(vi)	...	...	27	167	25	—
(vii)	...	...	33	215	20	12

Some physical properties of the solvents and solutions were measured to see if there was any correlation with absorption into mud. Viscosity was not a factor for, although cotton-seed oil is much more viscous than 1 : 1 mixture of cotton-seed oil and power kerosene, the absorption was about the same for each.

Surface tension was measured by the capillary rise method, and relationship, if any, to the degree of absorption was shown by the actual height attained in the capillary tube. Such a tube is a very simple model of the pores in the mud.

The capillary rise of both solvents and solutions was determined by the use of a piece of thermometer tubing. Results are given in the arbitrary divisions of the thermometer, the very narrow bore of which facilitated the taking of readings. A cathetometer was not available for determining the small differences in height which would have occurred in a tube of wider bore. Two pure liquids, benzene and ethyl acetate, were compared in the same tube in order to check the method. From the surface tension of benzene given in the literature, that of ethyl acetate was found to be 23·8 dynes per cm. at 25°C. The figure for ethyl acetate in the literature is 23·3 dynes per cm.

Solutions of DDT (83 per cent. pp') were prepared and the capillary rise measured. Results for extracts of D.929 (crude, neat, benzene hexachloride) with the solvents are included to show that the effect was the same for both insecticides. Column 4 gives the percentage recoveries of the solutions from surface layers of mud blocks (Table IV).

TABLE IV.

Capillary rise of solvents and solutions (at 25°C.).
(Thermometer tube readings.)

Solvent				Solvent alone	Solvent + 5% pp'DDT	Mean % of total dose in outer layer	Solvent + 5% extract of D.929
(i) ...	...	...	...	103·9	101·8	8	101·4
(vi) ...	...	...	...	101·1	99·2	13	—
(iii) ...	...	...	...	98·2	96·0	11	95·5
(ii) ...	...	...	...	97·5	95·5	13	95·2
(v) ...	...	...	...	93·8	92·1	19	—
T.P. 543 emulsion	...	...		—	88	35	—
Benzene	...	...	...	98·7			
Ethyl acetate...	...	...	81·0				

The addition of 6 per cent. technical DDT (5 per cent. pp') to the solvent reduced the rise by approximately two divisions while the extract from D.929 had slightly more effect.

If the values of percentage insecticide in the outer layer of mud are plotted against the heights of capillary rise, the graph forms a curve ; but although a relationship between the two seems evident, it is not a simple one. If it were so, a decrease of surface tension should decrease the degree of absorption. In an attempt to show this, the height of capillary rise of a 5 per cent. DDT (pp') power kerosene solution was lowered to 89 divisions by replacing 30 per cent. of the power kerosene by Triton X-100. According to the curve this mixture should have given an absorption equivalent to 30 per cent. of insecticide in the outer layer, but the actual figure was 11 per cent. as compared with 13 per cent. for power kerosene solution alone.

Further investigations on these or similar lines are required to ascertain the relationship, if any, between physical properties of solutions and their absorption by mud walls. For instance, the contact angles of the solution against mud should be measured but it was not found possible to do this.

Adsorption.

A few experiments were performed to see whether DDT was adsorbed by mud.

Using the sieved mud which is used for making blocks, four thimbles, or hollow cylinders open at one end, were made. These were partly immersed to a standard depth, in 5 per cent. pp′ DDT in power kerosene solution, in a closed vessel in such a way that the solution could reach the insides of the thimbles only by penetration through the walls. The kerosene travels through the mud very slowly and, if the surrounding air is not saturated with vapour, the solution evaporates as rapidly as it reaches the inner surface of the cylinder. Samples of the liquid which collects inside the thimble were taken for analysis from two of the thimbles after 4 days and from the other two after 7 days. The original liquid outside the thimbles was analysed at the beginning of the experiment and at the time of taking samples. The solution originally contained 5·00 per cent. w/v pp′ DDT. The results of the analyses were as follows :—

Days after immersion :	4	4	7	7
Thimble No.	1	2	3	4
(i) Per cent. w/v in solution outside thimble	5·04	5·04	5·06	·5·06
(ii) Per cent. w/v inside thimble ...	4·69	4·44	4·88	4·88

The differences between the concentrations inside and outside of the thimbles seem to indicate that adsorption takes place. Differences in degree are probably due to the variation in thickness of thimble walls.

Adsorption also occurred on the mud powder which is used for making the standard blocks. Ten ml. of 5 per cent. DDT in power kerosene solution were shaken occasionally for 1 hour with varying weights of the powder in test tubes. The contents of each tube were filtered and the concentration of DDT in the filtrate determined. The results were as follows :—

Tube No.	1	2	3	4	5
Gms. mud used	0	3	3	5	5
Mgms. present after filtration	504	500	499	494	496
Mgms. adsorbed per gm. of mud	—	1·3	1·7	2·0	1·6

The average number of mgms. adsorbed per gm. of mud is 1·7. This represents about 15 mgms. per sq. ft. in the outer layer of a block, and accounts for a high percentage of the amount found in the outer layer when normal dosages (100–200 mgms./sq. ft.) of DDT in oil solution are used.

Thus it seems that loss of insecticide is due both to absorption of the carrier oil and to adsorption of the insecticide from the oil to the particles of mud. The experiment was repeated using mud which had been heated to a dull red heat for two hours to destroy the organic material. Adsorption on to the heated mud was just the same as with unheated.

The phenomenon of adsorption may explain why the loss of insecticide activity on mud is not simply related to the physical properties of the solvents used. Further work is proceeding on the adsorption from different solvents.

Experiments with different concentrations.

Solutions of DDT in power kerosene were used at concentrations of 2 per cent., 5 per cent. and 10 per cent. w/v. Recoveries are given below. There was no significant difference either in the amounts recovered from the outer layers, or in the toxicities to test insects. Kills of *G. palpalis* after 15 mins. contact and of *A. aegypti* after contact of 1 hour were all very low.

Concentrations	Total DDT recovered mgms./sq. ft.	% in outer layer	Mean % in outer layer
2 per cent. ...	160, 167, 170, 172, 220	14, 19, 8, 11, 13	13
5 per cent. ...	143, 174, 177, 210, 214	13, 13, 12, 14, 12	13
10 per cent. ...	115, 179, 212, 279, 430	15, 14, 14, 11, 13	13

Experiments with different dosages.

A 5 per cent. DDT solution in power kerosene was applied to mud blocks at different dosages. The recoveries are given below and it will be seen that there was a trend towards greater absorption at the higher dosages. Although higher dosages can be obtained in the outer layer by making excessively heavy applications, the method leads to a further increase in wasted material. Kills were not markedly increased. For instance, a kill of only 40 per cent. was obtained when *G. palpalis* were exposed for 15 mins. to blocks with a total dosage of 1,035 mgms. per sq. ft. and 66 mgms. per sq. ft. in the outer layer.

Total dosage mgms. per sq. ft.	% in outer layer
143, 167, 174, 177	13, 14, 13, 12
208, 210, 214	14, 14, 12
376, 461, 486, 695, 769	9, 9, 11, 8, 9
933, 1,035, 1,199	7, 6, 10

Effect of repeated applications.

Mud blocks were sprayed three times, at weekly intervals, with a 5 per cent· DDT solution in power kerosene. At each application the attempted dosage was 200 mgms. per sq. ft. The percentage of insecticide in the outer layer was no greater after three applications than after a single application.

The total DDT recovered was 486, 590, 702, 728, 722 mgms. per sq. ft. with a corresponding percentage in the outer layer of 14, 12, 10, 9, 10.

Kills were low but increased slightly after each application. Twenty *G. palpalis* exposed for 15 mins. contact, showed a mortality of 15 per cent. after the first application, 25 per cent. after the second, and 40 per cent. after the third. Twenty-seven per cent. of 33 *A. aegypti* exposed for 1 hour to the same blocks after the third application were killed. Five hundred and ninety mgms. DDT per sq. ft. were recovered from the blocks used for these biological tests, and 73 mgms. DDT per sq. ft. of this was in the outer layer.

Effect of pre-treatment with water.

Five sets of mud blocks were sprayed with water which immediately soaked in. They were resprayed with water and then while the surface was still wet, sprayed with a 5 per cent. DDT solution in diesoline. Results obtained were compared with those from blocks treated at the same time with only a 5 per cent. DDT solution in diesoline. Pre-treatment with water had no effect either on the amount of absorption or on the toxicity to test insects. The amount of water required to fill up the air spaces in the mud would be very great.

Treatment	Total DDT recovered mgms/sq. ft.	Mean % in outer layer
DDT/Diesoline	149, 174, 191, 208, 256	8
Water+DDT/Diesoline ...	164, 167, 201, 203, 254	7

Effect of exposure of absorbed deposits.

It may be advantageous to have an insecticidal deposit through a certain depth of a mud wall, the outer surface of which may be continually powdering off with exposure of fresh insecticidal surface. Amounts of insecticide so exposed would, of course, be small.

A set of blocks was sprayed with a 5 per cent. DDT solution in power kerosene and the mean kill of two batches of *G. palpalis* exposed to them for 15 mins. contact was 6 per cent. An outer layer of 1·7 gms. weight, or approximately 0·15 mms. thickness, was scraped off and found to contain 51 mgms. DDT per sq. ft. Two further batches of flies were exposed to the new surface of the blocks for 15 mins. and the kill was 80 per cent. The blocks were then found to contain 371 mgms. DDT per sq. ft., of which 34 were in the outer layer of 1 gm. weight.

The increased kill obtained after the removal of the outer layer may have been due largely to the nature of the surface. The upper faces of the standard blocks used throughout the test were levelled off and were comparatively smooth. After a layer is scraped off the new surface is much rougher and consequently has a larger area exposed. Three sets of standard blocks, and three sets which had previously been scraped, were sprayed with a 5 per cent. DDT solution in power kerosene. There was no significant difference in absorption but blocks scraped before the application were more toxic, as the following figures show :—

Type of Block	mgms. DDT/sq. ft.		% mortality of *G. palpalis* after 15 mins. contact
	In outer layer	Total	
Standard	35	376	15
Scraped before application ...	37	292	40

Treatment with Emulsions.

The following concentrates were diluted 1 : 4 with water to give DDT emulsions.

(i) T.P.543 concentrate made by Technical Products, Ltd., and containing :—

Aromatic petroleum fractions	68·3%
Commercial DDT (85 per cent. pp')	17·7%
Emulsifiers or stabilisers	14·0%

(ii) T.P.543 concentrate with 8 per cent. w/v coumarone resin.

The proportions of DDT recovered from the outer layers of mud blocks and the toxicities to test insects were intermediate between those obtained from solutions and dispersible powders.(see Tables V and VI). The height of capillary rise of the T.P.543 emulsion diluted for spraying was 88 (see Table IV) and this corresponded closely to the amount of absorption. The addition of resin appeared to serve no useful purpose.

TABLE V.

Recovery of insecticide from mud blocks treated with DDT emulsions.

Formulation	Total dosage mgms. DDT/sq. ft.	% in outer layer	Mean % in outer layer
T.P. 543	211, 246, 248, 273, 274	37, 34, 44, 37, 21	34
T.P. 543+resin ...	162, 164, 198, 198, 216	33, 27, 22, 30, 24	27

TABLE VI.

Toxicity of blocks, treated with emulsions, to test insects and recovery of insecticide from the same blocks.

Formulation	mgms. DDT/sq. ft.		% mortality *G. palpalis* after contact of		% mortality of *A. aegypti* after contact of 1 hr.
	in outer layer	Total	15 secs.	15 mins.	
T.P. 543	57	274	25	75	69
T.P. 543+resin ...	44	198	5	50	43

Treatment with Dispersible Powders.

Water suspensions to contain 5 per cent. DDT w/v were made up from the following dispersible powders :—

*(i) Neocid B.A.50, manufactured by Geigy Co., New York, containing 5·3 per cent. hydrolysable chlorine and 38 per cent. para para isomer of DDT.

(ii) N210 spray powder, manufactured by Hygienic Chemical Co., London, and containing 1·96 per cent.* hydrolysable chlorine and 14·3 per cent. para para isomer.

(iii) Ditrene, manufactured by Technical Products Ltd., and containing 5·0 per cent. hydrolysable chlorine and 45 per cent. para para isomer.

The analytical figures were determined for the small samples used.

The suspended particles were largely filtered off at the surface of mud blocks and a white deposit was visible macroscopically. The surface deposits of DDT were higher than those obtained by applying oil solutions and emulsions, and as much as 77 per cent. of the total amount applied was recovered from the outer layer of a set of blocks treated with Ditrene. In the case of Ditrene, at least, the percentage recovered from the outer layer was increased with higher dosages.

In comparison with the low kills of G. palpalis and A. aegypti obtained after contacts of 15 mins. and 1 hour respectively with blocks treated with oil solutions and emulsions, kills on blocks treated with dispersible powders were remarkably high. Mortalities of from 75 to 100 per cent. were obtained after G. palpalis had made contacts of only 15 secs. duration, and mortalities of from 37 to 90 per cent. obtained after A. aegypti had made contacts of ½ hour (see Tables VII and VIII).

TABLE VII.

Recovery of insecticide from mud blocks treated with DDT dispersible powders.

Formulation	Total dosage mgms. DDT/sq. ft.	% in outer layer	Mean % in outer layer
Neocid B.A. 50 ...	77 100, 129, 161, 163	39, 43, 44, 54, 48	47
N210	180, 265, 269, 296, 357	62, 63, 61, 60, 62	62
Ditrene	39, 42, 52	46, 43, 46	45
Ditrene	233, 293, 525	73, 75, 77	75

TABLE VIII.

Toxicity of mud blocks, treated with DDT dispersible powder, to test insects and recovery of insecticide from the same blocks.

Formulation	mgms. DDT/sq. ft.		% mortality of G. palpalis after contact of 15 secs.	% mortality of A. aegypti after contact of	
	in outer layer	Total		½ hr.	1 hr.
Neocid	43	100	100	90	100
N210	111	180	100	60	100
Ditrene	24	52	75	37	—
Ditrene	169	233	90	56	—

*These figures were incorrectly quoted in Bull. ent. Res., 38, 1947, p. 345 as 53% and 19·6% respectively.—ED.

Two sets of mud blocks were treated with a water suspension of Neocid B.A.50, and kept in the laboratory. Flies and mosquitos were exposed to them at intervals over a period of ten weeks, after which time surface deposits were determined chemically.

The repeated applications of test insects and the container tubes to the blocks probably removed some of the surface deposits. Results were as follows :—

Time after application	1 day	1 week	2 weeks	4 weeks	8 weeks	10 weeks
Per cent. mortality of *G. palpalis* after 15 secs. contact	100	100	100	80	75	5
Per cent. mortality of *A. aegypti* after $\frac{1}{2}$-hour contact	75	70	50	38	—	0
DDT in outer layers	13	mgms. per sq. ft.				
DDT in whole block	68	mgms. per sq. ft.				

Treatment with Benzene Hexachloride.

The only benzene hexachloride preparation used in this series of tests was a dispersible powder D.P.530, manufactured by Imperial Chemical Industries, Ltd., and containing 50 per cent. benzene hexachloride and 6·5 per cent. gamma isomer, This was made up as a suspension in water to contain 0·5 per cent. gamma isomer w/v.

Surface deposits on mud were initially high, a mean of 76 per cent. of the total benzene hexachloride applied being recovered from the outer layer of blocks. There was, however, a rapid loss of insecticide, and the percentage in the outer layer decreased in 9 days to approximately 20 per cent. and in 24 and 33 days to 10 per cent. Toxicities to test insects decreased correspondingly. Details are given in Tables IX and X.

The variation in the dosages initially applied makes it impossible to draw definite conclusions as to the fate of the insecticide in the second or lower layer of the blocks. Loss of benzene hexachloride from the outer layer by volatilization was shown,

TABLE IX.

Recovery of benzene hexachloride from mud blocks at various times after the application of dispersible powder 530.

Days after application	Mgms./sq. ft. in outer layer	Mgms./sq. ft. in lower layer	Total dosage mgms./sq. ft.	Percentage in outer layer
0	96	28	124	77
	75	24	99	76
	99	30	129	77
2	94	77	171	55
	89	80	169	53
4	89	107	196	45
	17	54	71	24
9	17	66	83	20
	8	43	51	16
	12	60	72	17
15	10	44	54	19
	9	34	43	21
24	2·4	22	24	10
	2·7	23	26	10
33	2·6	21	24	11
	4	31	35	11

and reduction in the amount of surface deposits during the first week after applica'ion was obvious macroscopically.

TABLE X.

Toxicity of mud blocks treated with D.P. 530.

(a) To G. palpalis.

Blocks	Time after application	% mortality after contacts of				Benzene hexachloride mgms./sq. ft. recovered	
		15 secs.	2 mins.	5 mins.	15 mins.	in outer layer	Total
1	6 hrs. ...	100			-	96	124
2	6 hrs. ...	100					
	1 week ...	30	80	100		12	72
3	6 hrs. ...	100					
	1 week ...	35	80				
	2 weeks ...	—	55	70		10	54
4	6 hrs. ...	100					
	3 weeks ...	0		25		2·4	24
5	6 hrs. ...	100					
	3 weeks ...	0		20	40	2·7	26
6	6 hrs. ...	100					
	2 days ...	€0					
	4 days ...	65					
	1 week ...	10		65			
	2 weeks ...	0		20	30		
	3 weeks ...				30		
	4 weeks ...				25	4·0	₹5

(b) To A. aegypti after contact for ½ hour.

Time of application	No. of mosquitos	% mortality
6 hrs.	20	100
2 days	20	100
5 days	24	100
1 week	24	100
2 weeks	24	50
4 weeks	30	23

At the end of four weeks 2·6 mgms. benzene hexachloride per sq. ft. from the outer layer and 23·6 mgms. per sq. ft. from the whole blocks were recovered.

Meteorological conditions in the laboratory during these experiments were :—

	Mean daily temperature °F.		Relative Humidity	
	Maximum	Minimum	at 8.30 a.m.	at 2.30 p.m.
1st week	77·7	73·6	80	73
2nd week	77·7	73·1	75	74
3rd week	78·6	73·4	74	72
4th week	80·6	74·4	74	70

Applications to Vegetation.

Methods.

For the chemical estimations, leaves were first stripped with benzene to remove surface deposits. They were then dried, powdered and extracted in a soxhlet apparatus for the absorbed insecticide. It is evident that a solvent such as benzene will penetrate into the leaf during washing and remove interior DDT. To eliminate errors from this source the washing procedure was standardised by shaking the whole leaves with a given volume of solvent for a given time. A deposit entirely on the outside of the leaf can be obtained by slowly applying a known volume of DDT solution in ether from a pipette and directing a stream of air over the leaf so that the ether evaporates almost as soon as it touches the surface. Preliminary experiments showed that such a deposit on a leaf 30 sq. inches in area was completely removed by shaking with 20 ml. benzene for half a minute followed by a further 20 ml. for half a minute.

This procedure was adopted for all leaves of area up to 30 sq. inches (two washings of 30 ml. each were used for larger areas). DDT not removed by this washing but found in a soxhlet extract was considered to have been inside the leaf.

Examples where a further washing was interposed between the standard washing and the soxhlet extraction are shown in Table XI.

TABLE XI.

Type of leaf and spray	Mgms. DDT/sq. ft. leaf surface found in		
	1st Washing 2 × 20 ml.	2nd Washing 20 ml.	Soxhlet extract
Avocado pear			
(i) 5% DDT in kerosene and cotton-seed oil, 50 : 50	79	0	13
	88	0	15
(ii) Guesarol (DDT dispersible powder) ...	39	0	0
	63	0	4
(iii) T.P. 543 (DDT emulsion)	64	1	9
	81	0	10
Coffee			
5% DDT in kerosene and cotton-seed oil, 50 : 50	61	5	47
	45	5	67

The use of standard procedure, where it is known that the first washing will remove any outside deposit, ensures that the relative values found inside and outside the leaf are not affected by a variable amount of washing. Although the accuracy of the values found may not be equal for other spray formulations and other leaves, the amounts found inside the leaves are minimal quantities and serve to illustrate the extent of absorption. Certainly it seems unlikely that insecticide not removed by the benzene washings would be available to a resting insect.

All estimations of DDT and benzene hexachloride in the extracts are based on hydrolysable chlorine present, using the method described by Neal & others (1944).

Biological tests have consisted of simple contact exposures of laboratory bred *G. palpalis*, R.-D., to the treated leaf surfaces. Batches of 20 flies were exposed individually for 20 seconds to each treated leaf.

Initial penetration.

Young coffee plants were treated with a solution of 1 per cent. Waxoline Red in power kerosene at the rate of 2 qts. per 1,000 sq. ft. Penetration of the dye into

the substance of the leaf was immediately obvious, and irregular. Twenty-four hours later the surface of the leaf was cleaned, and crude transverse sections were cut. The cuticle of the upper epidermis was stained, and the dye could be seen in the palisade layer.

The initial loss of insecticide appears to be merely a result of direct and rapid penetration of the oil carrier through the cuticle of the leaf and this is borne out by the fact that this initial penetration varies considerably not only in the different types of leaves of different plant species but also in leaves of the same plant. It is impossible to repeat results even using plants of the same species and of the same age, presumably on account of the different surface structure and thickness of cuticle at the different ages of the leaves.

Loss of Insecticide from inside the Leaf.

In preliminary work a gradual disappearance of insecticide from the inside of the leaves was observed (Barlow & Hadaway, 1947, p. 337).

The following experiments were carried out to determine whether there was a transference of DDT from treated leaves to other parts of the plant.

(*a*) The upper surfaces of two leaves on each of several young potted coffee plants (*Coffea robusta*) were treated with a 5 per cent. DDT (pure) solution in 50 per cent. power kerosene and 50 per cent. cotton-seed oil at an estimated dosage of 100 mgms. per sq. ft. Two young leaves at the growing point and two or three below the treated leaves were left untreated. Application was made from a micropipette as evenly as possible and absorbent lint was tied round the petioles of the treated leaves to prevent creeping of the solution to the untreated parts of the plant. At various intervals after the applications quantitative chemical estimations of the DDT on the surface and inside the treated leaves were made.

The presence or absence of DDT in the untreated parts of the plant was determined qualitatively. A sample of the extract was evaporated to dryness in a $6 \times \frac{3}{4}$ inch test tube, 0·5 ml. of a mixture of equal parts concentrated sulphuric acid and fuming nitric acid were added and the tube heated in a water bath for 10 minutes. After cooling, the mixture was diluted with 1 ml. distilled water, cooled again, 1 ml. water added and then 2 ml. benzene. The acid was neutralized with solid sodium carbonate and the benzene layer withdrawn with a pipette and placed in a small test tube. Extraction of the aqueous layer was repeated with 1 ml. of benzene. On addition of 5 ml. of 5 per cent. potassium hydroxide in methanol a blue colour developed in the presence of nitrated pp'DDT. As pure pp'DDT was used in these absorption experiments there was no interference from other isomers. This method was not used quantitatively because a colorimeter with suitable filters was not available. Filters would have been essential as some constituents of the leaf extracts yield a yellow colour under these conditions and the colours obtained with small amounts of DDT are therefore green. Preliminary removal of the leaf pigments with activated charcoal does not prevent the appearance of the interfering colour.

A fifth of the soxhlet extract from each leaf or group of leaves was used for this qualitative test, the remainder being reserved in case the strength of the reaction should warrant a quantitative determination. Thus, as the method detects about 2 microgrammes or more of DDT in a sample, a negative result indicates that there is less than 10 microgrammes in the extract. The reactions obtained were classified as trace, strong and very strong.

An amount of 0·3 mgm. of DDT was found in the extract from three untreated leaves, below those treated, twelve days after the application of insecticide. Results are shown in Table XII.

TABLE XII.

Recovery of DDT from coffee plants.

(i) Quantitatively :—The figures per sq. ft. are accurate to 1 mgm. DDT.
In the extract of untreated leaves the accuracy is 0·1 mgm. ; 0 for untreated leaves means
that there was less than 0·1 mgm. present.

	Time after application	Treated leaves, DDT mgms./sq. ft.		Untreated leaves		
		On surface	Inside	At growing point	Others below treated leaves	Stem
Plant 1	1 day	Leaf (a) 40	35	0	0	0
		,, (b) 57	53			
Plant 2	4 days	,, (a) 66	47	0	0	0
		,, (b) 121	13			
Plant 3	7 days	,, (a) 84	24	0	0	0
		,, (b) 59	50			
Plant 4	12 days	,, (a) 50	67	0	0·3 mgms. in extract	0
		,, (b) 19	54			

(ii) Qualitatively.

	Time after application	Untreated leaves		Root and Stem	Leaf (b)
		At growing point	Others		
Plant 1	1 day	0	0	0	Very strong
Plant 2	2 days	0	Trace	0	,, ,,
Plant 3	7 days	0	0	0	,, · ,,
Plant 4	12 days	—	Strong	0	,, ,,

(b) No young coffee plants were available for tests to confirm the above result
and two young Avocado pear plants were used instead. Four leaves on each were
treated with 5 per cent. DDT (pure) solution in 50 per cent. power kerosene and
50 per cent. cotton-seed oil at an attempted dosage of 50 mgms. DDT per sq. ft.
Six to eight leaves at the growing point, and six leaves below the treated leaves
were untreated. The procedure was as in experiment (a).

Only a small proportion of the insecticide recovered was inside the leaves.
Traces of DDT were found qualitatively in untreated leaves of both plants and
0·3 mgms. were recovered in the extract from the leaves at the growing point of
Plant 1, fifteen days after the application of insecticide. Detailed results are
given in Table XIII.

Loss of Insecticide from the Surface of Leaves.

Deposits on treated vegetation are exposed to ordinary climatic conditions.
Loss of insecticide may be caused by the washing action of rain during heavy
showers and by the action of wind brushing the leaves one against the other.
The small-scale laboratory tests described below were carried out to compare the
persistence of deposits on leaves inside and outside the laboratory.

(a) The upper surfaces of six or seven leaves on a young potted Avocado pear
plant were treated ; the same number were left untreated. Two plants were treated
with a 5 per cent. DDT (pure) solution in 50 per cent. power kerosene and 50 per cent.
cotton-seed oil ; two other plants were treated with a 5 per cent. DDT emulsion
made from T.P.543 concentrate at an attempted dosage of 100 mgms. DDT per sq. ft.
One plant with each treatment was kept in the laboratory ; the other was kept

outside exposed to ordinary climatic conditions. Leaves were removed at intervals for biological and chemical tests. Corrections for the growth of the leaves are not made as this was negligible. Results are given in Table XIV.

TABLE XIII.

Recovery of DDT from Avocado pear plants.

(i) *Quantitatively.*

	Time after application				Treated leaves, mgms. DDT per sq. ft.		Untreated leaves	
					On surface	Inside	At growing point	Below treated leaves
Plant 1	15 days	...	...	... ...	Leaf (a) 15	4	0·3 mgms. in extract	0
					,, (b) 34	13		0
					,, (c) 45	5		
					,, (d) 47	16		
Plant 2	23 days	...	... ...	...	,, (a) 56	6	0	0
					,, (b) 39	9		
					,, (c) 48	10		
					,, (d) 31	6		

(ii) *Qualitatively.*

	Time after application					Untreated leaves	
						At growing point	Below treated leaves
Plant 1	15 days...	...	...	...	...	Very strong	Strong
Plant 2	23 days...	...	...	...	...	Trace	Trace

None of the leaves showed any immediate ill effects as a result of the treatment and there was no burning. A " tacky " film remained for three weeks on the leaves treated with the solution in power kerosene and cotton-seed oil, but this appeared to have been mostly washed off the plant kept outside after five weeks. A crystalline deposit from the emulsion remained on the leaves kept inside the laboratory for eight weeks, but was partly washed off leaves exposed outside during the first week. After three or four weeks, the two very young leaves at the growing point of both plants inside the laboratory shrivelled and became brown.

Records were kept both outside in a Meteorological Screen and in the laboratory. A shower (0·01 in.) occurred within 24 hours of the plants being exposed. Averages of daily readings are given in Table XV.

Treated leaves of plants kept inside the laboratory continued to be toxic for several weeks to flies exposed to them for contacts of only 20 seconds and eight weeks after treatment with T.P.543 emulsion a leaf gave a 75 per cent. kill. On the other hand, treated leaves exposed to weathering did not retain a high degree of toxicity for the same length of time. After only one week, kills on leaves treated with a solution of DDT in 50 per cent. kerosene and 50 per cent. cotton-seed oil fell from 100 to 15 per cent., despite the fact that approximately the same amounts of insecticide were recovered from the surfaces of the leaves. Surface deposits on leaves treated with emulsion appeared to be lost more readily as a result of exposure

to rain, but the residual insecticide retained its toxicity longer than in the case of treatment with oil solution.

(b) The upper surfaces of leaves of Avocado pear plants were treated with a water suspension of Guesarol dispersible powder containing 2 per cent. DDT at a dosage of 50 mgms. DDT per sq. ft. One plant was kept inside the laboratory; the other was kept outside. The water quickly evaporated leaving a white deposit on the surface of the leaves. The plants suffered no obvious ill effects as a result of the treatment. Heavy rainfall occurred during the first 24 hours of exposure, 0·87 inch falling in two hours, and almost the entire surface deposit was washed off the exposed plant.

TABLE XIV.

Toxicity of treated Avocado pear leaves to *G. palpalis*, and recovery of DDT from the same leaves. Twenty flies exposed for 20 seconds contact to each leaf.

Treatment	Area of leaf sq. ins.	Age of deposit	% kill in 24 hours	Recovery of DDT mgms./sq. ft.	
				On surface	Inside
Solution of 5% DDT in kerosene and cotton-seed oil 50 : 50. Inside	6·4	3 hrs.	100	54	50
	6·0	24 hrs.	100	87	15
	6·1	1 week	100	79	15
	4·3	2 weeks	100	121	14
	6·0	3 weeks	100	82	17
	7·0	5 weeks	10	34	9
Solution of 5% DDT in kerosene and cotton-seed oil 50 : 50. Outside	6·2	3 hrs.	100	79	13
	7·5	24 hrs.	100	56	8
	5·7	1 week	15	72	7
	5·3	2 weeks	10	76	13
	5·5	3 weeks	15	58	20
	6·2	5 weeks	0	11	11
T.P.543 emulsion. Inside	6·0	3 hrs.	90	71	15
	4·5	24 hrs.	90	92	13
	6·0	1 week	95	64	3
	5·5	2 weeks	95	60	13
	5·3	3 weeks	95	64	11
	5·9	5 weeks	70	67	4
	4·0	8 weeks	75	87	17
T.P.543 emulsion. Outside	7·5	3 hrs.	80	65	1
	9·7	24 hrs.	50	81	10
	7·0	1 week	75	32	1
	8·2	2 weeks	50	28	2
	6·9	3 weeks	40	32	9
	8·6	5 weeks	15	25	1
	7·5	8 weeks	15	25	5

The deposit persisted on the leaves kept in the laboratory for several weeks but very little DDT was recovered from the inside of the leaves. That remaining on the surface was much less toxic to flies than an equivalent dosage of insecticide applied as a solution or an emulsion. It is probable that the large amount of inert material making up the bulk of the dispersible powder masks the effect of the insecticide. The results are given in Table XVI.

This test was started one week before those previously described, so the meteorological records given in Table XV apply for most of the experiment.

TABLE XV.

Meteorology.

(i) *Outside.*

	Temperature °F.		Relative humidity			Sunshine in hours per day	Rainfall	
	Max.	Min.	6 a.m.	12 a.m.	6 p.m.		Total ins.	No. days
1st week	75·1	63·3	96·0	76·0	82·0	4·0	0·86	2
2nd ,, ...	76·7	63·0	96·5	81·6	82·4	7·0	0·56	2
3rd ,, ...	77·5	62·7	95·1	76·4	82·1	5·7	0·50	2
4th ,, ...	75·7	62·8	96·5	77·3	84·3	6·1	0·29	2
5th ,, ...	76·5	64·4	92·1	74·0	79·0	7·3	0·52	3
6th ,, ...	77·4	64·4	96·0	78·1	77·4	8·9	1·30	4
7th ,, ...	79·7	64·1	94·0	70·6	81·9	6·6	1·20	1
8th ,, ...	79·2	63·8	96·8	75·3	85·3	6·8	2·30	2

(ii) *Inside laboratory.*

	Temperature °F.		Relative humidity		
	Max.	Min.	6 a.m.	12 a.m.	6 p.m.
1st week	76·0	71·1	84·7	82·0	78·7
2nd ,, ...	77·3	72·4	80·4	79·6	77·3
3rd ,, ...	76·3	71·4	81·6	82·0	79·4
4th ,, ...	76·4	71·6	82·3	80·6	79·9
5th ,, ...	77·4	74·1	81·4	76·6	75·4
6th ,, ...	78·3	73·7	80·0	79·4	75·7
7th ,, ...	78·4	73·7	78·9	76·3	76·3
8th ,, ...	79·1	73·6	79·7	77·6	76·7

TABLE XVI.

Toxicity of Avocado pear leaves, treated with Guesarol wettable powder, to *G. palpalis* and recovery of DDT from the same leaves. Twenty flies exposed for 60 seconds contact to each leaf.

Treatment	Age of deposit	% kill in 24 hours	Recovery of DDT mgms./sq. ft.	
			On surface	Inside
Guesarol. Inside	3 hrs.	25	43	0
	24 hrs.	20	63	4
	1 week	20	56	13
	2 weeks	15	78	7
	3 weeks	10	53	17
	8 weeks	20	41	21
Guesarol. Outside	3 hrs.	20	39	0
	24 hrs.	0	7	7
	24 hrs.	0	0	10
	1 week	0	26	14
	2 weeks	0	5	2

Loss of Toxicity of exposed Surface Deposits.

The results of preliminary work showed little correlation between the amount of insecticidal deposit on the surface of the leaf and its toxicity to flies. This is again evident from the results given in Table XIV. A deposit of 72 mgms. DDT per sq. ft.

was recovered from the surface of a leaf treated with a solution of DDT in 50 per cent. kerosene and 50 per cent. cotton-seed oil after it had been exposed to ordinary climatic conditions for only one week. Yet this gave a kill of only 15 per cent. as compared with 100 per cent. kill on a leaf, kept inside the laboratory, carrying a surface deposit of 79 mgms. per sq. ft.

The following small-scale tests were made to determine the effect of continuous exposure to sunlight on deposits of DDT.

(a) Three glass plates, 6 inches square, were placed side by side in a measured area of ground which was then sprayed with a 5 per cent. solution of DDT (83 per cent. pp′) in power kerosene with a small Four Oaks hand pressure pump. One plate was kept inside the laboratory, one outside in all weathers day and night, and the other outside in the sun only. The last-mentioned was brought into the laboratory at night and when rain threatened.

Laboratory bred flies (G. palpalis) were exposed to the treated surfaces individually for 20 seconds, 12 hours and 12 days after the application of insecticide. Kills are given in Table XVII.

TABLE XVII.

Age of deposit	Treatment of deposit	No. of flies exposed	% kill in 24 hours
12 hours	(i) In laboratory	20	100
	(ii) Outside, all weathers	20	100
	(iii) Outside, sun only	20	100
12 days ... ·... ...	(i) In laboratory	20	95
	(ii) Outside, all weathers	20	0
	(iii) Outside, sun only	20	10

After a few heavy showers the deposit had been washed almost completely from the glass plate kept outside in all weathers. When the exposures of flies had been completed, twelve days after the application of insecticide, the glass plate (iii) was placed outside for a further exposure to sunlight. Unfortunately a sudden shower spoilt the deposit for chemical determination, but DDT was recovered from the plate kept inside at the rate of 144 mgms. per sq. ft.

The deposit kept outside in the sun only was exposed to 48 hours of sunshine.

	Screen	Inside Laboratory
Mean daily Maximum Temperature	77·4°F.	79·8°F.
Absolute Maximum Temperature...	79·5°F.	85·0°F.
Mean daily Minimum Temperature	64·8°F.	73·3°F.
Absolute Minimum Temperature	61·0°F.	70·0°F.
Mean daily R.H. at 12 noon	80%	80·5%

(b) Dosages of 25 and 50 mgms. pure DDT per sq. ft. were applied to glass plates by spreading evenly over them measured amounts of a 1 per cent. DDT solution in power kerosene from a micropipette. Some plates were exposed to sunlight and some kept in the shade in the laboratory. G. palpalis were exposed to the treated surfaces for 15 seconds, twelve hours after the application of insecticide and seven days after exposure to sunlight. Results, given in Table XVIII, show that the deposits exposed to sunlight were less toxic than those kept inside the laboratory.

TABLE XVIII.

Dosage	Treatment	Age of deposit	No. of flies	% kill in 24 hours
25 mgms./sq. ft. ...	Inside	12 hours	20, 20	90, 85
	Outside	,,	20, 20	90, 90
	Inside	7 days	20, 20	85, 85
	Outside	,,	20, 20	10, 15
50 mgms./sq. ft. ...	Inside	12 hours	20, 20	100, 100
	Outside	,,	20, 20	100, 100
	Inside	7 days	20, 20	100, 95
	Outside	,,	20, 20	15, 25

During the seven days, the glass plates were exposed to 53 hours of sunshine. The sun was bright and hot for periods of seven or eight hours a day.

	Screen	Inside Laboratory
Mean daily Maximum Temperature	79·1F°.	78·7°F.
Absolute Maximum Temperature...	80·0F°.	80·0°F.
Mean daily Minimum Temperature	64·5F°.	74·4°F.
Absolute Minimum Temperature	63·0F°.	73·0°F.
Mean daily R.H. at 12 noon	74·1%	77·3%

Accurate measurement of the temperature reached on the surface of the glass plates was not possible but a crude method showed it to be of the order of 50–60°C. Pure and commercial DDT is only decomposed at relatively high temperatures according to Balaban and Sutcliffe (1945).

Microscopic examinations showed a marked difference between the deposits kept in the laboratory and those exposed to the sun. The solution applied to the plates kept inside the laboratory remained in the form of droplets, each a supersaturated solution of DDT in kerosene. After a week small masses of needle crystals had formed from a few of the small droplets, but the majority were still liquid. Crystallisation of DDT from the larger droplets did not take place until 2–4 weeks after application. When the droplets were scratched with a needle crystallisation followed and the same result was obtained as a result of contact between the droplet and the tarsus of a fly walking over the surface. On the other hand, the droplets on the glass plates exposed to the sun formed hard resinous masses after a short time. When these were scratched with a needle crystallisation did not take place, the hard mass merely breaking into smaller pieces.

The four plates exposed to sunlight had a total area of 1 sq. ft. and an estimated deposit of about 37 mgms. pp'DDT. The plates were individually washed with benzene and the combined washings made up to 250 ml. One 50 ml. sample was analysed for hydrolysable chlorine, a second for total chlorine and the remaining extract evaporated to dryness and the residue crystallised from alcohol. The same procedure was adopted with the other set of four plates kept in the laboratory.

Hydrolysable chlorine was determined by the procedure previously described (Neal & others, 1944). Total chlorine was determined by the method of Smith and Stohlman (1944), using sodium and alcohol. After evaporation of the benzene a dark brown viscous liquid was left which was dissolved in a little alcohol and filtered hot to recover pp'DDT. After cooling, the crystals were filtered and air dried. There seemed to be some impurity present in both extracts and two recrystallisations

were necessary. These further manipulations probably explain to some extent the low recoveries. The melting points of the crystallised material and the mixed melting points with pure pp′DDT were determined.

Results were as follows :—

	Plates in Laboratory	Plates in Sun
(1) Mgms. DDT on the plates indicated by the hydrolysable chlorine content	29·0	20·5
(2) Ratio of hydrolysable chlorine to total chlorine	0·201	0·214
(3) Percentage recovery of pp′ DDT from 3/5 of the extract*	29	33
(4) Melting point of recovered DDT	108°C.	107–108°C.
(5) Mixed melting point of recovered DDT with pure pp′ DDT	107–108°C.	107–108°C.

*The hydrolysable chlorine content found in (1) indicates the presence of 17 mgms. in the remaining extract used for recrystallisation from the laboratory exposed plates and 12 mgms. for the sun exposed plates. The total quantities recovered are expressed as percentages of these figures.

A significant chemical change on the sunlight exposed plates was the increase in the ratio of hydrolysable to total chlorine. The differences between 0·214 and the theoretical value (0·200), and the value of the laboratory exposed plates is much greater than the experimental error of the method of analysis. The significance of this increase is not yet understood but it is evident that there has been no loss of hydrochloric acid from DDT to form the ethylene derivative as this reaction would result in a decrease of the ratio. Recently Fleck (1949) has shown that DDT on exposure to ultra-violet light undergoes a reaction under anaerobic conditions which would give an increase in the ratio of hydrolysable to total chlorine.

Although the observed difference in physical structure of the two deposits seems sufficient to explain the loss of toxicity it appears that there is also some chemical change when DDT deposits from oil solution are exposed to sunlight.

Summary.

Formulations of DDT have been applied to standard mud blocks. Toxicities to *Glossina palpalis* and *Aëdes aegypti*, and the proportions of insecticide recovered from an outer layer of approximately 0·1 mms. thickness have been compared.

Absorption of DDT in oil solutions was considerable, and amounts recovered from the outer layer were only from 8–19 per cent. of the total dosage applied. Adsorption of the insecticide from the oil on to mud occurred. There was some correlation between the capillary rise of the solvent and the extent of absorption. Toxicities of the blocks to test insects were low.

Using concentrations of 2 per cent., 5 per cent. and 10 per cent. in power kerosene, there was no increase in the proportion held on the surface. Application of excessively heavy dosages increased the amounts in the outer layers but there was a trend towards greater absorption, and therefore to greater waste, at the high dosages. Repeated applications similarly built up a larger dosage in the outer layer but did not increase the proportion there.

Emulsions were intermediate between solutions and wettable powders as regards absorption and toxicity.

Up to 77 per cent. of the insecticides applied as wettable powders was recovered from the outer layer, and toxicities were correspondingly high. When benzene hexachloride wettable powder was used there was rapid loss of benzene hexachloride

from the surface by volatilisation. After fifteen days the dosage had decreased considerably and the percentage in the outer layer had fallen from over 70 per cent. to 20 per cent.

Loss of DDT by penetration of the carrier oil through the leaf cuticle may occur when an oil solution is sprayed on to vegetation. The extent of penetration varies with different plants.

There is an indication that small amounts of DDT are transferred from the inside of treated leaves to other untreated parts of young coffee and Avocado pear plants.

Deposits of DDT on leaves exposed to ordinary climatic conditions remained toxic to tsetse flies for a longer period when applied as an emulsion than as an oil solution. Deposits from a water suspension of a wettable powder were washed off readily by rain.

There is some evidence that continuous exposure to sunlight produced some chemical change in, and reduced the toxicity of, DDT deposits applied to glass plates as a solution in kerosene.

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OBSERVATIONS ON THE CONTROL OF KENYA COAST *GLOSSINA*.

By J. Y. Moggridge.

Department of Tsetse Research, Tanganyika Territory.

(Plate V.)

Control by Undercutting of Thicket.

A study of the data provided by fly rounds showed that *Glossina pallidipes*, Aust., *G. brevipalpis*, Newst., and *G. austeni*, Newst., thrive in those areas of the various vegetation types that provide the relatively densest vegetation. This knowledge was applied in a small reclamation experiment.

An area of 92·4 acres of thicket growing on coral rag rock on the Kenya coast was isolated from the surrounding thicket by barrier clearings during the dry season of 1938. The clearings on the north and south sides of the experimental block were 140 yards broad while that on the west side was 200 yards broad. The whole of the coral rag thicket area east of the experimental block had been cleared to a distance of 1,100 yards in order to make a landing ground.

Owing to shortage of labour, the work of making the barrier clearings (small as they were) progressed so slowly that regeneration in the clearings made first had rendered them useless, but all the clearings were finally completed and regeneration was cut back to some extent.

During the wet season (April to July) regeneration grew strongly and the clearings became largely ineffective against *G. pallidipes* (Pl. V, fig. 3 shows the clearing at the time of cutting).

The clearings having been roughly completed by December 1938, catches started in the experimental block on 20th January 1939, and the last catch was made on 28th July 1939. A short control fly round was carried out from October 1938 to July 1939 in the coral rag thicket immediately north of the experimental block. Catches were made in the control in the early forenoon simultaneously with catches in the experimental block. On the control round one bait ox and two catchers were used, whilst in the experimental block, in order to ensure that tsetses did not escape capture, two bait oxen and five catchers were used.

The method employed in reclaiming the experimental block was under-cutting of the thicket. Close growing thicket and climbers (other than lianes) and woody growth were cut out but shrubs and trees were left untouched. When the under-cutting had been completed the resultant trash was piled at convenient distances and especially over the stumps of thicket patches. As soon as it was dry it was set alight and burnt fiercely, scorching the leaves of nearly all trees and shrubs in the vicinity and charring the stumps and exposed roots over which the fire was burning. The resultant leafless and shadeless state of the canopy brought about a marked intensity of the dry season conditions within the experimental block ; for example, the mean saturation deficit during catches made in March was 5·3 mb. in the control and 12·3 mb. in the experimental block. When the under-cutting was completed it was possible to walk freely in the block and, apart from widely separated clumps of grass, the floor of the block was bare. Later in the season grass grew strongly and succulents started regenerating. This regeneration had to be cut back at the end of the wet season. In practice, however, the grazing of cattle and small stock in the reclaimed area would keep regeneration in check. Photographs illustrating the clearing are shown on Plate V.

Comparative results between the catches in the experimental block and in the control are set out in Table I.

TABLE I.

Average catches per hour in the experimental block and its control during the period January to July 1939.

Dry season (" clean " barrier clearings).

25th January to 28th March (inclusive)	Total *pallidipes*	Total *austeni*	Total *brevipalpis*	Number of catches	Average time taken per catch
Experimental block	0·44	0·40	0	9	59 minutes
Control	32·6	51·4	2·8	7	44 minutes

Wet season (" dirty " barrier clearings)

April to July (inclusive)					
Experimental block	10	0	0	15	36 minutes
Control	82	30	0·71	14	40 minutes

During the wet season, regeneration in the clearings developed unchecked. The table shows the increased catches made in the experimental area during the wet season. There can be no doubt that the regeneration in the narrow clearings aided the passage of *G. pallidipes* from the heavily infested thicket to the north. During the dry season at a time when the barrier clearings were reasonably clean *G. pallidipes* virtually disappeared from the experimental area. Both *G. austeni* and *G. brevipalpis* were eliminated.

Fly rounds carried out on the actual site of the experiment between January and June 1937 showed mean apparent densities of 3 and 18 per hour, respectively, for *G. brevipalpis* and *G. austeni* before the experiment started, while *G. pallidipes* showed a mean apparent density of 70 per hour.

No animals of any sort were seen in the experimental block, and spoor was rarely seen, after under-cutting of the thicket had been completed.

In practice it will probably be found uneconomical to undercut large areas of homogeneous thicket or forest to provide grazing for stock. The method is more likely to be applied to areas of grazing which are unsafe for stock on account of the presence of tsetses in clump thickets within the grazing. The open nature of such an area renders undercutting and the construction of barrier clearings a relatively inexpensive undertaking.

The writer wishes to stress that the reclamation experiment described above suffered from inadequate protective clearings. The tree-supporting barriers were unreasonably narrow and the frequent passage of natives and motor vehicles down the middle of two of the barrier clearings were sufficient to militate against any prospect of eradicating *G. pallidipes* from the experimental block.

The experiment indicates that *G. austeni* and *G. brevipalpis* can be eliminated from coastal thicket by undercutting the vegetation and that there are reasons to believe that by the same method *G. pallidipes* also may be eliminated.

Observations on the Use of Traps against *G. pallidipes*.

In the course of the foregoing experiment, an attempt was made to determine whether traps would be efficacious against this species under Kenya coastal conditions.

Five S.S. traps (Swynnerton, 1933) and 1 Harris trap (Harris, 1930) were used in trapping experiments during March, April and May 1939. They were placed in pairs in the savannah, coral rag thickets and clump thickets and their catching sites

TABLE II.

Trapping results compared with control catches in three types of vegetation.
Dry season—March and April 1939.

Trap number and vegetation type	Traps					Controls					
	Hours of catching	Average catch per hour		Average female percentage		Average catch per hour 6–9 a.m.		Average catch per hour over 12 hours		Average female percentage over 12 hours	
		pallidipes	*austeni*	*pallidipes*	*austeni*	*pallidipes*	*austeni*	*pallidipes*	*austeni*	*pallidipes*	*austeni*
Savannah 1 ...	603	1·22	0·02	85·0	64·2	150	29	91	13	37·2	52·9
,, 2 ...	560	0·93	0·02	82·4	69·2						
Coral rag thicket 3 ...	537	0·27	0·02	76·7	53·9	—	—	28	29	31·8	37·3
,, ,, 4 ...	503	0·44	0·06	83·3	63·3						
Clump thicket 5 ...	551	0·34	0·07	81·1	77·8						
,, 6 ...	540	0·24	0·05	77·9	82·7						

changed frequently. The tsetses were removed daily in the evenings after a light flitting of the wire retaining-cages, and the catches for each trap were recorded separately.

The traps were sited near fly rounds so that these and all-day catches carried out in their vicinity might act as controls. In the savannah and the coral rag thicket special catches were made in the immediate vicinity of the traps. After these catches the traps were moved and re-sited, so that there should be no suggestion that they were left to catch in a caught-out area.

In the savannah, two African children catching off an ox between 6 a.m. and 9 a.m. in the dry season were able to catch more *G. pallidipes* in 30 seconds than the best trap was able to capture in 60 minutes (Table II). Also the children were able to catch in 2 minutes as many *G. austeni* as No. 1 and No. 2 traps combined caught in 50 hours of daylight. In addition, two children caught off an ox for 10 minutes each hour throughout the day, as described below in the case of the coral rag thicket, and these figures are also given in the table.

In the coral rag thicket, two African children catching off an ox throughout the day for 10 minutes hourly were able to catch as many *G. pallidipes* in 2 minutes as No. 4 trap was able to capture in 2 hours and as many *G. austeni* in a similar time as this trap was able to capture in 20 hours of daylight. These catches were made in close proximity to the traps. The clump thicket had no control, but experience had shown that this type of country would provide catches of 37 per hour at this time of year.

Table III repeats for the wet season the results obtained in the dry season. No *G. brevipalpis* were caught in the traps, and only one in 12 hours in the savannah, in the wet season only.

TABLE III.

Trapping results compared with control catches in three types of vegetation
Wet season—May, 1939.

Trap number and vegetation type	Hours of catching	Traps				Controls			
		Average catch per hour		Average female percentage		Average catch per hour over 12 hours		Average female percentage	
		pallidipes	*austeni*	*pallidipes*	*austeni*	*pallidipes*	*austeni*	*pallidipes*	*austeni*
Savannah 1 ...	369	0·3	0·06	86·5	68·1 ⎫	114	10	36·2	56·4
„ 2 ...	369	0·6	0·03	82·7	90·8 ⎭				
Coral rag thicket 3 ...	328	0·7	0·02	84·2	60·0 ⎫	76	20	38·2	32·1
„ 4 ...	333	0·2	0·05	83·5	61·1 ⎭				
Clump thicket 5	399	0·3	0·03	84·1	81·1 ⎫	—	—	—	—
„ „ 6	295	0·1	0·02	94·3	33·3 ⎭				

It should be noted that although the female percentages of the trap catches are considerably higher than those taken by hand yet the relative inefficiency of the traps is such that actually more females would be caught in the hand catches.

Summary.

A small reclamation experiment staged in the coral rag thicket under unfavourable conditions proved successful in eradicating *Glossina austeni* and *G. brevipalpis* and in virtually eradicating *G. pallidipes*. Applied with adequate protective clearings, it is thought that the method may prove entirely successful in eradicating *G. pallidipes* as well as *G. austeni* and *G. brevipalpis*.

Under both dry and wet season conditions traps proved quite ineffective.

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Fig. 2. Portion of the coral rag thicket after the under-cutting of the under-growth.

Fig. 4. Typical grass growth on the floor of the thicket of the experimental block during the rainy season. The white object in the centre of the photograph to the left of the African assistant is a piece of creeper caught in the branches of a tree. It has escaped burning and has not yet rotted sufficiently to fall to the ground.

Fig. 1. Representative portion of the coral rag thicket before reclamation measures were applied.

Fig. 3. Portion of the clearing at the time of cutting; edge of the treated thicket in the background.

THE TAXONOMIC STATUS OF *DROSICHA STEBBINGI* (GREEN) AND *DROSICHA MANGIFERAE* (GREEN) (HEM., COCCID.).

By Lieut.-Commdr. ABDUL LATIF, M.Sc. (Agric.).

Associate Professor of Entomology, Punjab Agricultural College, Lyallpur, Pakistan.

Green (in Stebbing, 1902) described a species collected by Stebbing at Dehra Dun in 1899 as *Monophlebus stebbingi*.* The principal diagnostic character of this species was the presence of six fleshy tassels at the caudal extremity of the male (fig. 1, *a*). Green (in Stebbing, 1903) proposed the name *Monophlebus stebbingi* var. *mangiferae* for a form found on mango in the Shalimar Gardens near Lahore in which the adult male carried eight fleshy caudal tassels. Later (1908) he described material from the Shalimar Gardens on mango as *Monophlebus stebbingi* var. *octocaudata*.

Green suggested (1924) that "if, however, the name *Monophlebus* should be rejected for our large Oriental species, they would fall naturally into *Drosicha* of Walker, which comes next on the priority list, and of which the type specimen (a female) is available for study." He also gave a tentative synopsis of the characters of the females of five species, including *stebbingi* and *octocaudata*. Morrison later (1928) accepted the inclusion of the species from eastern and south-eastern Asia and adjacent areas in the genus *Drosicha* and placed *Drosicha octocaudata* as a synonym of *D. mangiferae*.

Food-Plants.

Beeson (1941) states that *D. stebbingi* is mainly a pest of forest trees like Sal (*Shorea robusta*) while *D. mangiferae* is a pest of fruit trees. Beeson is so definite on the point that he places literature dealing with *D. stebbingi* from mango under *D. mangiferae*. Hingston (1929), Richards and Sharma (1934) and Misra and Rao (1938) have recorded *D. stebbingi* from fruit trees (mango) in Central Provinces, Cawnpore and Benares, respectively. Rahman & Latif (1944) have stated that fruit trees such as mango are the common host plants of both *D. mangiferae* and *D. stebbingi* and that the latter is by no means confined to forest trees. This confusion necessitated a re-examination of the problem.

Adult Males in Nature.

The identification of the male of *D. mangiferae* and *D. stebbingi* is based, as already pointed out, on the presence of eight and six fleshy tassels respectively. A large number of newly emerged males collected from different orchards were examined for caudal tassels. They exhibited an almost continuous series from complete absence of the fourth pair of tassels to its full development (fig. 1, *a-e*).

The following stages can be clearly recognised :—

(*a*) Only three pairs of abdominal tassels.

(*b*) Fourth pair of tassels rudimentary, represented by a small knob only.

(*c*) One of the fourth pair of tassels slightly developed and the other rudimentary.

(*d*) Fourth pair of tassels short.

(*e*) Fourth pair of tassels fully developed.

Hingston's (1929) illustration of *D. stebbingi* shows a fourth pair of tassels corresponding to the above-mentioned category (*b*) or (*d*).

*This species was originally described under the name of *Monophlebus stebbingii*. The spelling of the specific name was emended to *stebbingi* in the Errata.—Ed.

It has been further observed that males of any two or three of the above-mentioned categories may copulate, one after the other, with a single female.

a.   **b.**

c.   **d.**   **e.**
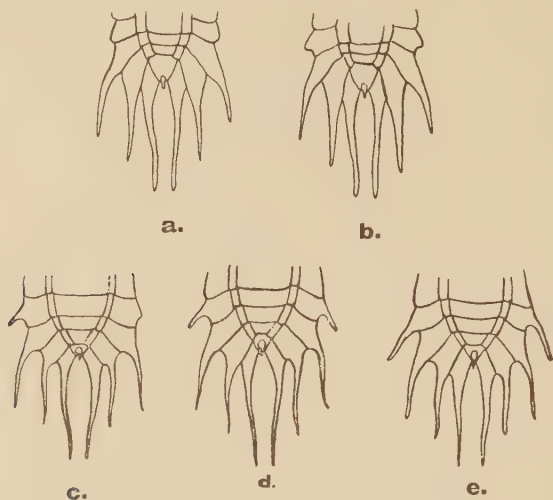
Fig. 1.—Variation in abdominal tassels of *Drosicha stebbingi.*

Adult Males bred in Laboratory.

To obtain more precise information, 376 pupae were collected in April, 1940, from a mango orchard at Lyallpur and the males that emerged were classified according to the above-mentioned five types.   Out of 85 males, 10, 32, 18, 18 and 7 fell into categories (*a*), (*b*), (*c*), (*d*) and (*e*), respectively.   This shows that there is a great deal of variation in the development of the 4th pair of abdominal tassels and that males showing variation in the development of this pair constituted 80 per cent. of those which emerged from pupae collected from the same mango garden.

Examination of Antennae of Nymphs.

The antennae of the first-instar nymph of *D. stebbingi* has five joints (fig. 3, *a*). Lefroy (1908) and Beeson (1941) state that in *D. stebbingi* var. *octocaudata* (=*mangiferae*) the first-instar antenna has six joints.

a.

b.

c.

d.
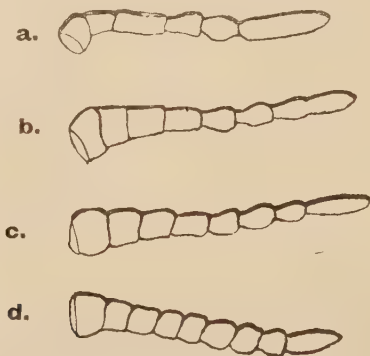
Fig. 2.—Antennae of *Drosicha mangiferae* (after Lefroy, 1908).   (*a*) 1st instar ;   (*b*) 2nd instar ♀ ;   (*c*) 3rd instar ♀ ;   (*d*) 2nd instar ♂

According to Morrison (1928), however, in the genus *Drosicha* the antenna of the first-instar nymph is five-jointed. Observations made by the writer show that the 1st, 2nd and 3rd-instar female nymphs and the adult female of *D. mangiferae* have 5, 6, 7 and 8-jointed antennae, respectively, as against 6, 7, 8 and 9 joints, respectively, mentioned by Lefroy (1908) ; third-instar male nymphs have 9-jointed antennae.
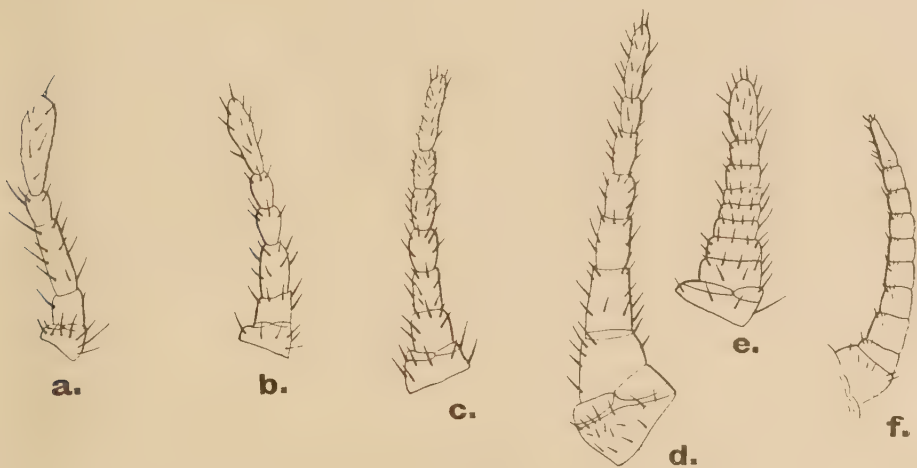
Fig. 3.—Antennae of *Drosicha stebbingi.*   (*a*) 1st instar ;   (*b*) 2nd instar ;   (*c*) 3rd instar ♀ ;   (*d*) adult ♀ ;   (*e*) 3rd instar ♂ ;   (*f*) pupa.

The extra joint in the first instar of *D. mangiferae* mentioned by Lefroy (1908) and again by Beeson (1941) as the only feature distinguishing *D. stebbingi* and *D. mangiferae* in this instar appears from Morrison's (1928) and the writer's observations to be non-existent.

Nymphs bred in Laboratory.

During 1941, 219 females copulating with eight-tasseled males and an equal number of females copulating with six-tasseled males were collected. The females subsequently oviposited normally. The eggs of the two batches were kept in separate dishes at 25°C. from May to November, when they hatched out almost simultaneously. From each lot 640 nymphs were examined to determine whether there was any character by which the two batches could be differentiated, but no such character could be found.

Conclusions.

Drosicha mangiferae (Green) and *D. stebbingi* (Green) have food plants and habitats in common and the same number of antennal joints in all nymphal instars. No character differentiating them exists in any nymphal instar. Intermediate conditions of the caudal tassels of the males occur and males that differ from one another in the degree of development of the variable fourth pair of tassels will copulate with the same female. A form of the male having a small fourth pair of tassels was referred by Hingston (1929) to *D. stebbingi,* and it is highly probable that *D. mangiferae* (Green) is a synonym of *D. stebbingi* (Green).

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A SIMPLE METHOD FOR THE ESTIMATION OF CONTACT INSECTICIDES.*

By B. J. Krijgsman and Nelly E. Berger.
Laboratory of Comparative Physiology, University of Utrecht.

The great increase, in recent years, in the number of contact insecticides has created a demand for methods whereby the content of active substance in samples of these insecticides may be estimated quantitatively in a simple manner. Such methods are important, because on the one hand the producer wishes to know if his product meets the necessary requirements and on the other hand the official services concerned with the control of insect pests must be able to test the fundamental value of commercial preparations. Moreover quantitative methods for the estimation of contact insecticides are useful for scientific research in this field.

The analytical chemical method is usually the obvious way of determining the content of the active principle, but in some instances it is cumbersome, costly or difficult to carry out. There are, for example, the isomers of hexachlorocyclohexane, which are very difficult to determine chemically. The chemical determination of rotenone and allied active substances in derris root is also not simple. In these cases it is better to replace the chemical test by biological methods, in which the action of the preparations upon a suitable insect species is compared with that of a standard preparation.

Biological estimation of insecticides is sometimes objected to on the grounds that only one species is used as the test object and that the results give no indication of the action upon insects with different susceptibility. It must be remembered, however, that these estimations do not claim to give indications of differences in sensitiveness and do not give full information of usefulness in the field. The biological estimation is often only an equivalent of the chemical analysis and thus is only a measure of content of the active substance. The insect used as a test object takes the place of the chemical indicator. For this reason it is necessary to carry out the experiments with one species in order to keep the indicator constant.

Biological test methods depend upon the exposure of a given number of insects to the action of a given quantity of the preparation for a given time. Though this principle is very simple, the application of it involves difficulties. Large numbers of a suitable insect, capable of being easily cultivated under standard conditions all the year round, must be available and an accurate dosage of the insecticide must be possible. That the latter is not easy is obvious from the literature, in which methods are described for dosing accurately by dusting, spraying, submerging, etc. (Hewlett 1947, Parkin & Green 1943, Tattersfield & Potter 1943, Tilemans 1941). These methods have not led to standardisation ; each research centre uses its own method and test animals, and consequently the results are not generally mutually comparable. It is not the intention here merely to add another to the many methods already known, but to make an attempt to introduce a standard method that would be useful all over the world, even in laboratories with the simplest equipment, for the comparative determination of the fundamental activity of contact insecticides and of their residual action.

The well known grain weevil, *Calandra granaria*, cultivated in wheat at 25°C., was chosen as the test object. The culture originated with one male and one female, the offspring of which served as test material. This ensured that the results were not affected by strains with different susceptibility.

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After comparing the various methods of applying the insecticide to the weevils, the most accurate procedure appeared to be to allow the weevils to walk on a solid and uniform film of the preparation, the quantity of which, available to the weevils per sq. cm., was known. After many trials the following experimental arrangement was decided upon.

Petri dishes having a diameter of 9 cm. and a height of 1·5 cm. were used. Other dimensions, provided they are the same in every test, can naturally be used. Most contact insecticides are soluble in volatile organic solvents, such as acetone. One cc. of such a solution was introduced into the petri dish and by continuous movement the solution was spread as evenly as possible over the bottom and sides until the solvent had evaporated, leaving a solid film of insecticide on the glass. In the same manner the inside of the cover (not the sides) was treated with 1 cc. of the solution. Fifty weevils were then introduced into the dish and, after the cover had been put in position, placed at 25°C. This gave the weevils 170 sq. cm. of insecticide film over which they walked at a brisk pace, travelling over the bottom, the sides and the inside of the cover. Any source of error due to the possible unevenness of the film was eliminated by this mobility. After 60 minutes the weevils were transferred to a clean petri dish, again placed at 25°C. and after three days the mortality determined. Only those animals which could no longer make any movement whatever, were considered dead. Four or five different concentrations were used in each of the sixteen experiments carried out and as many control tests with weevils in clean dishes. From the mortality in the latter tests the real mortality of the treated animals was then calculated by Abbott's well-known formula ; the percentage mortality is given by $\dfrac{a-b}{a} \times 100$, a being the percentage survival in the controls, b that in the treated animals. For each concentration the quantity of insecticide per sq. cm. was calculated. Table I gives the results of a complete series of experiments with the pure γ-isomer of hexachlorocyclohexane, in which $\dfrac{8}{17}$, $\dfrac{6}{17}$, $\dfrac{4}{17}$, $\dfrac{2}{17}$ and $\dfrac{1}{17}$ microgram of the γ-isomer was present per sq. cm. The mortality is given in percentages.

TABLE I.

$\frac{8}{17}$ microgram	$\frac{6}{17}$ microgram	$\frac{4}{17}$ microgram	$\frac{2}{17}$ microgram	$\frac{1}{17}$ microgram
88	82	74	51	41
90	78	63	60	27
82	82	76	57	34
91	73	78	34	30
92	74	80	66	47
90	78	76	64	79
86	88	52	60	25
84	78	50	64	51
81	74	60	46	39
75	69	71	56	24
62	73	76	50	58
64	86	63	36	40
88	80	72	59	36
80	77	80	65	42
90	81	80	48	28
86	84	76	52	41
Average mortality : 83%	79%	70%	54%	38%

The average mortality at the various concentrations may be used for the construction of the mortality curve, each point of which is determined by the average of sixteen tests (800 weevils). The LD 50 is generally used as the point of comparison, and this may be determined by interpolation, but as the mortality curve is sigmoid, interpolation is not easy. By means of Bliss' probit method (Bliss, 1935, 1935a), the sigmoid may be transformed into a straight line (i.e. between mortalities of 40 and 90 per cent.). The average mortalities at the concentrations used in Table I, using the probit method, are plotted in fig. 1. From this it is easy to determine the LD 50 for the pure γ isomer of hexachlorocyclohexane as $0 \cdot 1 \pm 0 \cdot 005$ microgram per square centimetre. This figure is completely relative, but wholly reproducible. If the LD 50 of preparations containing an unknown percentage of γ-isomer is determined, the γ-isomer content of the unknown preparation can be calculated by comparison with the LD 50 of the pure isomer.
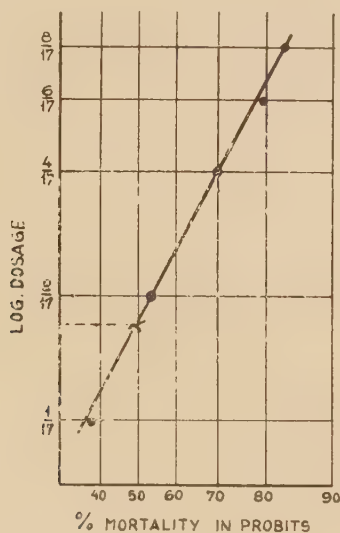
Fig. 1.

This method is also useful for the determination of the contact action of DDT preparations, in which the procedure is exactly the same. Pure p,p′DDT under the test conditions appeared to have an LD 50 at $6 \pm 0 \cdot 3$ microgram per sq. cm. and Velsicol 1068 at 2·4 micrograms per sq. cm.

Several contact insecticides adhere very strongly to glass and careful cleaning is therefore necessary. It is sufficient in the case of hexachlorocyclohexane and Velsicol 1068 to rinse the used dishes with acetone, and after washing thoroughly with soap and water to rinse them again with acetone. This is not sufficient for dishes treated with DDT which, having been washed with acetone, must be allowed to stand overnight in kerosene ; they are then washed with soap and water and rinsed again with acetone.

Whilst this method is satisfactory for comparing different preparations of one and the same insecticide, a comparison of the results with different insecticides is not always correct. DDT, for instance, works relatively slowly and a longer experimental time than three days might be necessary if the action is to be compared with that of other insecticides. Nevertheless, the mortality after three days does

not differ materially from the final mortality and it may, therefore, be concluded that the γ-isomer of hexachlorocyclohexane under the given conditions is many times more toxic to the weevils than DDT. It has been proved that DDT and the γ-isomer of hexachlorocyclohexane are about equally toxic when injected into insects (Dresden & Krijgsman, 1948), and consequently the difference in toxicity in the contact action must be due to the difference in the rates at which the skin is permeated.

The above method may naturally be used under variable conditions. The total duration of the test, for instance, may be varied, but a longer period than five days is not to be recommended as the mortality in the untreated dishes becomes too high. Paralysed animals may be counted as half dead and the time of exposure can be changed ; if necessary, it can be allowed to equal the total experimental time. In this way, less rapidly penetrating insecticides may be tested. Insecticides that penetrate slowly cannot, however, be tested by this method, because they do not poison the insect in the available test time, in spite of the fact that the insecticide is present in excess. Weevils, for instance, can be made to walk about for several days on a thick film of pure rotenone without any appreciable mortality resulting. In these cases, results might be achieved if test animals with a more permeable skin were available.

Another limitation is that insecticides having a fumigant action during the time of exposure cannot be used, as the LD 50 is the result of the combined contact and fumigant actions and the extent of the latter action is not known. Moreover, it should be emphasised once more, that the results obtained have an exclusively relative value. Variation of the test conditions also changes the LD 50, but if these, once chosen, are kept constant, the method is useful for comparative research and may, it is believed, sometimes successfully replace chemical analysis.

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NOTES ON EAST AFRICAN BUSH LOCUSTS WITH SPECIAL REFERENCE TO *PHYMATEUS AEGROTUS* (GERSTAECKER 1869) (ORTH., ACRID., PYRGOMORPHINAE).

By D. Keith McE. Kevan.

Department of Zoology, School of Agriculture, University of Nottingham ; late Entomologist, Department of Agriculture, Kenya.

The name, Bush Locust, was used by Bishop (1940) for the South African species, *Phymateus leprosus* (Fab.), which he studied in some detail. As understood in the present paper, the name is applicable to any species of the genus *Phymateus*, or other large Pyrgomorphine, in which the nymphal (or hopper) stages remain gregarious throughout life, but excludes solitary species and species of such genera as *Zonocerus* and *Taphronota*, in which the younger nymphal instars only may be gregarious for a limited period.

Phymateus aegrotus (Gerstaecker).

(a) Synonymy and Distribution.

This striking species was originally described by Gerstaecker (1869 & 1873) from southern Somaliland as *Poecilocera aegrota*. *Phymateus hildebrandti* described from Somaliland by Brunner von Wattenwyl (in Bolívar, 1884) is undoubtedly a synonym (**syn. nov.**) although the two species are treated as distinct in the same work by Bolívar whose conception of *Phymateus aegrotus* at that date, however, appears to have been erroneous.

The adult has been figured by Bolívar (1909) and (as *P. hildebrandti*) by Paoli (1934), who also mentions it under both names from " Italian " Somaliland. Schulthess (1895 & 1898) recorded it (as *P. hildebrandti*) from the Ogaden and (1898) from southern Ethiopia (now in Borana Province) while Rehn (1901) and Salfi (1939) have reported it from eastern Ethiopia (Gallaland) and from Ethiopian, Moyale (on the Kenya-Ethiopia border), respectively.

Jannone (1945) refers to what may be the same species from Eritrea. This he calls " *Phymateus hildebrandti* or a congeneric species near to it " which will be referred to later in this paper as *Phymateus* sp. The only Eritrean specimens of the genus known to the author (1♂, 1♀, Arafali, Eritrea, in the collection of the Royal Scottish Museum, *ex* Berlin Museum) belong to the very similar species, *P. pulcherrimus*, I. Bol., which is known also to the writer from Ethiopia and to which further allusions are made later in this paper.

P. aegrotus is a common species in Kenya, occurring in numerous localities from the southern border to the extreme north, whence it extends into Ethiopia, and from altitudes of a few hundred to over 5,000 feet. It is, apparently, more confined to the eastern half of the Colony, although Chopard (1921) records it from Sotik to the south-west as well as from the Southern Uaso Nyiro in the south, and the majority of the records are from dry grass and thorn-bush country. Dogiel and Sokolov (1916) also mention the species from British East Africa under the name of *P. hildebrandti*.

Sjöstedt (1909) recorded *P. hildebrandti* from northern Tanganyika and it is stated by Bolívar (1884) to occur in South Africa, but the latter record undoubtedly refers to another species.

From personal observation or from specimens, *P. aegrotus* is known to the author from numerous localities in east, north-east and north Kenya, from southern Ethiopia, from the Ogaden and from Somalia, while from information received and specimens in the British Museum, it also appears to be common in British Somaliland.

(b) *Economic Status and Plants attacked.*

A feature which many, if not all, species of *Phymateus* have in common is that, while the adults are solitary and usually of little or no economic significance, the nymphs form bands. These are similar to those of true locust hoppers and, although smaller, can be of local importance as agricultural pests.

In *P. aegrotus*, as with other species, there appear to be outbursts of greater activity from time to time but, in the intervening periods, the species is uncommon if not actually rare. Although scattered adults have been found in various parts of eastern Africa every year from 1938 to 1947 inclusive, hopper bands were only known to occur in the middle of 1938, the middle of 1943, at the end of 1945 and in 1946–47. It seems unlikely that, had there been any in the intervening periods, they would have been entirely overlooked by the large Locust Control forces operating over the whole area during the latter half of the period in question. Jannone (1945) reported hoppers of *Phymateus* sp. near Adi Ugri (14° 52′N., 38° 50′E.) in Eritrea in July 1944, but the climate, topography, etc., of that country have little in common with those of East Africa as a whole.

It seems fairly clear from the existing records that hopper bands occur only at two periods in the year corresponding to the rainy season—from May until July (1938, 1943, 1947), and from November to January (1945–46, 1946–47). Adults have been found in almost every month of the year but only in large numbers in July and August (1943, 1947).

As an agricultural pest, *P. aegrotus* has usually been of local importance only since the hoppers do not usually occur in cultivated areas. The adults, particularly the females, are too sluggish to present a threat to other areas by mass migration even if it was their habit to remain gregarious after passing from the hopper stage. It is not certain even that the gregarious state is always adopted by the hoppers.

Jannone (1945) records damage to market garden crops by adults and hoppers of *Phymateus* sp. in Eritrea. Apart from this record, the only cases where notable crop damage is so far known to have occurred are in the Isha Baidoa area (03° 07′N., 43° 38′E.) of Somalia in May–June 1943, the Moyale area on the Kenya-Ethiopia border in May–July 1947 and in the Machakos area of Kenya in June and July of the same year. This damage was quite considerable in places where the laziness or absence of the plot-owners allowed the hoppers to develop unmolested. Tomatos, potatoes, sweet potatoes, young maize, native spinach and lettuce, as well as cabbages and other brassicas, suffered particularly. Older maize and cucurbits of various kinds were damaged to a lesser extent while beans and pigeon pea were more or less unmolested. In the Isha Baidoa infestation millet (*Sorghum*) suffered some damage, but there was little else available in the way of crops.

In uncultivated areas the hoppers appear to be polyphagous, grasses and low bush alike being eaten. There is, however, a marked preference for certain Euphorbiaceae and perhaps other latex-producing plants. Young, newly-planted, *Euphorbia* hedges, of a species unknown, were completely eaten out in Moyale, according to the District Commissioner. In captivity, the hoppers feed well on the leaves of *Euphorbia splendens* Bojer.

Other East African species which are known to form hopper bands and which may damage crops are *P. pulcherrimus* I. Bol., *P. purpurascens* Karsch and *P. viridipes* Stål. The first mentioned species appears to be confined to the highlands of Ethiopia and Eritrea while the other two are more generally distributed in tropical

Africa ; in Kenya they are more frequently met with in cultivated areas at higher altitudes than *P. aegrotus* although they occur also at lower elevations where the humidity is higher and the vegetation denser. *P. viridipes* is known locally in Kenya as the Green Coffee Locust since it sometimes causes damage to coffee plantations. It has also recently (June 1947) been found attacking and damaging a crop of lavender (*Lavandula vera* D.C.) near Nanyuki (00° 01′N., 37° 05′E.).

P. pulcherrimus has been observed to cause considerable damage, in the hopper and the adult stages, to garden plants in Addis Ababa. Dr. H. B. N. Hynes has very kindly supplied this information and states that adults attack radish, carrot, lettuce and " nasturtium ".

(c) Life-history.

So far as is known, no large concentrations of adults have been observed immediately prior to the appearance of hopper bands and the indications are that the eggs remain in the ground for some considerable time before hatching with the onset of the rains. In the case of *P. leprosus* (Fab.), which has been more fully studied than other species, the eggs are reported to remain in the soil for about nine months in South Africa (Bishop, 1940).

There are five nymphal instars in the male and six in the female. Direct evidence to substantiate this by cage rearing is lacking in the case of the female but the adults of the two sexes show such a marked difference in size that it is almost certain that this is the case. Further evidence is provided by the fact that the male hoppers of the fifth instar are similar in size to those of the *penultimate* female hopper stage but are more advanced in the development of the wing-rudiments. This difference in wing-bud development coupled with similarity in size first becomes noticeable in the fourth instar (see also Coleman, 1911 ; Coleman & Kunhi Kannan, 1911 ; Kevan, 1943 ; Uvarov, 1928).

The interval between hatching and the appearance of the imago is ten to twelve weeks (Moyale ; April to July 1947) but the duration of the individual stadia is unknown. After the final moult, which took place at Moyale in July 1947, not long after the end of the rains, the adults remained in the area for some little time and then dispersed.

It is not known when mating takes place or if a reconcentration of pairing individuals occurs as in the semi-gregarious Indian species, *Aularches miliaris* (L.) (Hutson, 1926 ; Uvarov, 1928). It seems likely that mating takes place before dispersal, the resulting eggs remaining dormant in the soil until the next rains as with *P. leprosus* (Bishop, 1940). Jannone (1945) states that there is a loose reconcentration of adults (*Phymateus* sp.) in the Eritrean highlands early in the rains followed by mating and oviposition. It may be that something similar also occurs in East Africa proper since *P. pulcherrimus* in Ethiopia appears to have similar habits (see p. 362). There is, however, the possibility that Jannone was in fact dealing with that species and not with *P. aegrotus*.

P. leprosus apparently breeds only every other year, its egg and hopper stages being of long duration (Bishop, 1940), but *P. aegrotus* breeds at least once, and probably twice, a year in East Africa, the two generations corresponding to the two rainy seasons. In the Eritrean highlands, where there is one rainy season only, from July until about the end of September, there is a single generation of *Phymateus* sp. ; a period of about eight or nine months (from October until June) elapses before the adults attain sexual maturity (Jannone, *l.c.*). From this one would deduce that the egg and hopper stages together last from three to four months.

It would seem that there is no particular preference of soil type for oviposition but if there be any, it is for heavy " black cotton " soil. Jannone (*l.c.*) notes that the eggs of *Phymateus* sp. are laid in open clayey terrain.

Notes on *Phymateus pulcherrimus* I. Bolivar in Ethiopia.

The following field notes on the occurrence of *P. pulcherrimus* in Addis Ababa have been very kindly supplied by Dr. H. B. N. Hynes. This species is very similar to *P. aegrotus*. Final instar nymphs, of which the males were much smaller than the females, and a few teneral adults were observed two weeks after the end of the rainy season on 22nd September 1943. Many small hopper bands were also reported by natives at slightly lower altitudes (about 8,000 feet) at Casa Incis and Old Ghebbi in lower Addis.

Adults were observed flying singly and sluggishly about the city and feeding on various plants, from the end of November, 1943, until the beginning of March, 1944. Hoppers were also seen 30 miles north of Addis Ababa on 9th January, 1944. The rainy season began on 1st March and a few pairs of *Phymateus* were seen copulating. The females had become very gravid by 16th March and were unable to fly ; many were also very battered, particularly about the wings.

Six females were examined (all with full guts) and it was found that, in the ovaries, each follicle contained a single egg but all the eggs were in the same stage of development. The eggs averaged between 7 and 8 mm. in length, apart from one specimen in which they measured 5 mm. An egg count of four of the specimens revealed 202, 172, 220, and 132 eggs per gravid female, each ovary of a single individual having approximately the same number of eggs (63 and 69, and 109 and 111).

Although there were still several mating pairs on the following day, many of the females were obviously spent and with traces of froth on the ovipositors. An examination of a further 10 ovaries showed that six of these were spent, and of the remaining four, two had eggs of 5 mm., one of 4 mm. and one of 7 mm. in length. The last mentioned had 91 and 99 eggs in the individual ovaries. All the specimens had full guts and the spent females, apparently, were not developing further eggs although on 25th March spent females were still active and feeding, which might suggest further egg development.

Large numbers of adults were still to be observed in the lower part of the city on the 19th March, but only one individual was seen above 9,000 feet. Later, (30th April, 1944), they were observed quite commonly above this altitude.

Throughout April, there were heavy rains and *Phymateus* (females particularly) became much scarcer. Sporadic pairing, however, occurred but no hoppers appeared. On 30th April, a number of males and a few females were still to be seen ; three females and twelve males were all observed within a few yards of each other. One isolated female was found to be digging with her ovipositor in sandy soil between stone road sets. There were no signs of other such holes, but there had been much rain.

P. pulcherrimus became distinctly rare by 10th May. It was still wet and there was no sign of hoppers, from which it was deduced that there must be a prolonged diapause—a similar conclusion (using the term in the wide sense) to that reached by the author in respect of *P. aegrotus* and in accordance with what occurs in *P. leprosus* (Bishop, 1940). It was unfortunately not possible to determine whether this is the case, or whether the development of the eggs is merely very slow, since Dr. Hynes was transferred from Addis Ababa on 21st May, but up to that date, no hoppers had appeared. The author considers from this evidence that diapause more probably takes place in the adult as in *Phymateus* sp. in Eritrea (Jannone, 1945), lasting from shortly after one rainy season at the end of September, when teneral adults were observed, until the beginning of the next at the beginning of March, when mating was seen. The period required for maturation in this instance would be about six months, depending on climatic conditions.

Reflex Actions in *Phymateus* spp.

P. aegrotus, in common with other members of the genus, possesses the faculty of producing a pungent, offensive secretion by a process of autohaemorrhage from special gland-like structures at the base of the hind femur. The offensive smell produced has earned for *P. leprosus* the Afrikaans name of " Stink Sprinkaan ". The process of autohaemorrhage in *P. aegrotus* has been studied by Pavlovskiĭ (1916) and is discussed by Uvarov (1928). It appears to render these insects repulsive to birds and other vertebrates which do not normally feed on them. The same is true of other species of *Phymateus* such as *P. purpurascens* Karsch and *P. viridipes* Stål and certain other Pyrgomorphine genera including *Zonocerus* (Carpenter, 1946).

Autohaemorrhage has also been observed by the writer in the *hoppers* of *P. aegrotus* and *P. viridipes* which are capable of producing a similar strong smell when seized and which are apparently also repugnant to most birds ; even lizards have not been seen to feed on them in nature. The fluid, both in the adults and hoppers of these two species, may sometimes be forcibly ejected for several centimetres when the insect is held.

According to Dr. Hynes autohaemorrhage takes several forms in *P. pulcherrimus* adults. On every occasion observed, frothy bubbles exuded from the base of the abdomen near the first abdominal spiracles and often from behind the posterior coxae. In some specimens the " bleeding " was far more extensive than in others, and it was thought that it may come from behind the middle and posterior angles of the abdominal sterna I to V or VI ; one female produced these frothy bubbles at the base of the hind wing and from an unidentified source near the tympanum or possibly from the posterior thoracic spiracle. One male ejected a jet of blood, about 10 centimetres long, from under the pronotum. Dr. Hynes was, however, unable to induce this reaction a second time.

A second defensive or warning device adopted by *P. aegrotus* is a vivid colour display resulting from the raising of the wings (Kevan, 1947). *P. purpurascens* has been observed by the author to display the same characteristic and Carpenter (*l.c.*) also makes a brief mention of it when referring to probably the same species. Dr. Hynes notes that *P. pulcherrimus* when roughly handled will raise the wings in a similar manner to a butterfly and arch the abdomen, thus fully displaying the bright vermillion hind wings in a similar manner to that described for *P. aegrotus* (Kevan, *l.c.*). Usually the action is said to be followed by autohaemorrhage. They also defaecate and, in common with almost all Acridids, pour out a brown fluid from the mouth, the continued production of which results in a clearer liquid.

Hopper Bands of *P. aegrotus* (Gerst.).

The hopper-bands are of extremely small size when compared with those of true locusts, but are unlike those of certain other allied genera such as *Zonocerus* and *Taphronota*, the hoppers of which remain clustered on vegetation in dense masses in the younger stages, dispersing later. They retain the gregarious state and marching habit throughout their lives and have thus occasionally deceived locust control organisations in East Africa. They do not, however, march with the same vigour or regularity as true locust hopper-bands and are inclined to loiter in one area although travelling quite rapidly when on the move. The bands become rather long and narrow on the march, somewhat reminiscent of processionary caterpillars, and remain very densely crowded. They do not appear to march in the heat of the day and normally cover short distances only. The hoppers are usually found on bushes, when at rest, but this is not an invariable rule, as some remain on the ground.

The hoppers are very voracious feeders and the vegetation in the path of an advancing band may be eaten down almost to ground level ; the grass in such cases

looks almost as if it had been cut with a scythe and certain bushes and small trees are completely stripped.

Very few observations have been made on the bands of hoppers but they vary in size from patches of a few square feet to over twenty square yards. Of 33 bands of marching fourth- and fifth-instar hoppers observed by the author in the Moyale area in June, 1947, only four were more than five square yards in extent, the remainder varying between less than one and just over four square yards. Larger bands than those seen by the author have been reported in the same area at the same time and the largest of these were said to be up to 35 square yards and over.

The usual size of the resting bands observed was about one square yard, but a single marching band often split up to " rest " and was represented by more than one such patch. The hopper density in marching bands was not estimated but in most instances it was not apparently very much less than in resting bands where concentrations of 60 to 80 fourth- and fifth-instar hoppers per square foot were observed.

Marching and stationary bands, unlike those of true locusts, are not greatly disturbed by the near approach of moving objects, human or otherwise. This may be because they rely on devices other than rapidity of movement to protect them from their enemies.

The area normally infested by hopper-bands seems to be small and limited to, at most, a few square miles although there is not sufficient evidence to be certain of this point. The infestation centred on Moyale, on which the above observations were made, extended over a very large area (through about $2\frac{1}{2}°$ latitude and about $1°$ longitude), both in northern Kenya and southern Ethiopia. The intensity of this infestation, however, was patchy and variable, being as much as three to five or more bands per acre in the vicinity of Moyale itself and only about one or two bands per mile traverse in the lower country to the south-east and very patchy to the south. A rough estimate of the average band-density was 30 per square mile in the centre of the infestation.

According to Dr. Hynes, *P. pulcherrimus* also behaves in a gregarious manner in the hopper stage, all the hoppers marching in the same direction after the manner of locust hoppers, but more sluggishly and in very small bands (those observed only consisted of 200 to 300 final-instar nymphs). Feeding by hoppers of this species occurred at all hours of the day in Addis Ababa but it is not clear if this implies that marching also took place at the same time. The writer assumes that it does so, since *P. aegrotus* hoppers were not observed to feed to any great extent, except while on the march.

Control Measures.

Poison bait is effective but the bands are of such small size that they do not warrant the use of this method. The most satisfactory means of dealing with the hoppers of *P. aegrotus* is probably to beat out the bands with branches. Once a plot in a heavily infested area has been cleared, it can be protected from reinfestation by digging a shallow trench round it such as was formerly used in the control of locust hoppers (see Uvarov, 1928, for details). Light hand-applications of seven per cent. di-nitro-ortho-cresol are also effective.

Description of Hoppers of East African *Phymateus* spp.

Hoppers of the various species of the genus known to the author are all very similar but they may be distinguished one from the other by their coloration, particularly in the later instars. The earlier stages are less readily separated.

Adequate descriptions of the hoppers of *P. aegrotus* (Gerst.), *P. viridipes* Stål and *P. purpurascens*, Karsch, have not previously been made. The following detailed notes on the coloration of the final-instar nymphs of these three species are given to

supplement the key to the species given later (p. 366). The hoppers of *P. pulcherrimus* I. Bol. are not described as the writer has not had an opportunity of studying living material but it may be said that they present a much more purplish appearance than that of any of the other species.

(a) *P. aegrotus.*

The basic coloration is coal-black, but the whole hopper is flecked and spotted with white or yellowish-white maculae which, with the exception of the smallest, have bright orange-red centres, giving the hopper a very striking and distinctive appearance in life. The preserved insect loses a great deal of contrast in colour.

The following is a more detailed description of the coloration of the final instar in which the flecks and maculations are as stated above.

Antennae : Unspotted. *Head* : Labrum with a large central confluent pair of spots ; clypeus with a pair of small widely-separated flecks ; frons with a pair of semi-colon-shaped marks, each with a small spot above it ; eyes grey-brown encircled by small flecks, those directly above the eyes being very much the largest and irregularly confluent with a pair of large spots on the occiput ; cheeks with one anterior and two posterior maculae and two minute flecks interpolated within the triangle so formed ; frontal carinae between the eyes yellowish-white ; mandibles pitchy brown.

Thorax : Margins of pronotum with small (often confluent) flecks all round ; prozona on each side with four (sometimes three) large spots, the uppermost of which is on the large anterior dorso-lateral boss and the lowest of which is elongated from front to rear and situated near the lateral margin ; mesozona on each side with four rather smaller spots behind and slightly below those of the prozona ; metazona with a pair of anterior and a pair of posterior dorso-lateral spots, and on each side, about the middle of the postero-lateral margin, a rather large elongated macula. Several other small flecks are scattered here and there among the spots described. The markings of the pronotal bosses along the dorso-lateral lines tend to be confluent, almost giving the appearance of stripes continuing on to the head as far as the eye. Mesopleuron with one large and two small spots ; metapleuron with two stripes. Prosternal tubercle orange or yellowish-white ; meso- and metasterna with four large, irregular, paired, orange and white blotches. *Wing Rudiments* : Anterior pair black ; posterior pair black with orange-red tracheae.

Legs : All coxae and trochanters, apices of femora and bases of tibiae, purple-red (not blue as in the adult) ; anterior and middle femora and tibiae with numerous small flecks, carinae streaked yellowish-white ; posterior femora with all carinae yellowish-white and a row of eight to ten yellowish-white spots on the basal half of the externo-median area ; posterior tibiae with carinae and spines yellowish-white ; all tarsi black with greyish pulvilli.

Abdomen : Dorsal line orange, narrowly bordered yellowish-white ; each tergum laterally (except for the last two) with a small anterior spot and a very large posterior semi-lunar patch (the latter extending on to the hind margin and with a very prominent orange centre), and, near the lower margin, a small anterior and a small posterior spot ; each sternum with the posterior margin narrowly bordered yellowish-white (wider laterally, the middle line of the last sternum also whitish) and anteriorly with a pair of large, lateral, semi-lunar spots (extending to the pale posterior margin of the previous sternum). Ventral valves of the rudimentary ovipositor of the female hopper with a lateral spot at the base.

In earlier instar hoppers the maculations take the same general form and distribution but are proportionately smaller, the penultimate instar being very similar to the last but the youngest stages have orange-red (not orange and white) spots and black (not coloured) anterior wing rudiments, trochanters and femoro-tibial joints.

(b) P. viridipes.

Black, whole underside suffused yellow, maculations yellow, of varying sizes.

Antennae : Unspotted. *Head* : Eyes brown. Clypeus and mouth-parts scrawled with yellow ; frontal carinae yellow ; frons with a pair of orange-red spots on lower margin, otherwise with a few minute yellow dots ; cheeks with one red spot and numerous irregular minute dots ; vertex with two yellow dots ; occiput with two pairs of orange-red maculae.

Thorax : Bosses of pronotum red or orange-red, pro-, meso- and metazona each with a pair of orange-red maculae on either side, rest of pronotum with numerous small, scattered, yellow dots. Pleura spotted with orange-red and yellow. Sternum with two pairs of large, orange-red blotches. *Wing Rudiments* : Both pairs of wing buds black with yellow tracheae.

Legs : Two anterior pairs of legs irregularly streaked and spotted yellow, tarsi and hind tibiae unspotted ; hind femora with carinae yellow and a row of yellow spots along the externo-median area. Tibial spines yellow ; coxae, trochanters, femoro-tibial joints, dull purple.

Abdomen : Dorsal line orange-red, narrowly bordered yellow ; a row of large lateral abdominal spots and a pair of large blotches on each abdominal sternum orange-red ; last two abdominal segments and genitalia suffused and scrawled yellow ; rest of abdomen with yellow spots of varying sizes.

(c) P. purpurascens.

Black, head, thorax, abdomen and legs densely spotted with minute, yellow-green dots of even size.

Antennae : Unspotted. *Head* : Eyes reddish brown ; a row of six spots below the eyes and across the frons, bright red.

Thorax : Bosses of pronotum and about six lateral pronotal spots, one spot on the mesopleuron, a row of four spots on the metapleuron and four spots on the metasternum, all bright red. *Wing Rudiments* : Both pairs of wing-buds black with yellow-green tracheae.

Legs : Spotted, tarsi and hind tibiae unspotted ; externo-median carinae of hind femora, bright red ; remaining femoral carinae and tibial spines yellow-green ; coxae, trochanters and femoro-tibial joints dull red or reddish brown.

Abdomen : A row of large bright red lateral spots along the dorsal line and a pair of large blotches of a similar colour on each sternum.

The hoppers of this species are rather stouter in appearance, although a little smaller, than those of the other species described. The overall length of the hoppers varies with the degree of development of any given instar and is of little value as a measurement, but final instar male hoppers of *P. aegrotus* and *P. viridipes* when alive are about 45 mm. long ; females are about 60 mm. Unfortunately, no measurements of more reliable characters such as femur-length are available.

Key to the East African Species of *Phymateus.*

ADULTS.

I. Olive-grey ; bosses of pronotum dull purplish red ; tegmina blue or purple, speckled yellow ; hind wings bright orange-red, speckled black ; abdomen blue or purple, ringed yellow ; legs olive-grey, coxae and knees blue or purple.

    (a) Tegmina and abdomen blue and bright yellow ; coxae and knees blue ; hind wings unspotted in the basal third of the anal field..*P. aegrotus* (Gerst.)

    (*b*) Tegmina and abdomen dull purple and yellow ; coxae and knees dull purple ; hind wings spotted almost all over (the spots differing from the last in relation to cross-veins)...........*P. pulcherrimus* I. Bol.

II.   Green ; bosses of pronotum red, green or blackish ; tegmina green, unspeckled ; hind wings not as above ; abdomen green, sometimes ringed light yellow-green ; legs (including coxae and knees) green.

    (*a*) Bright green to light grey-green ; bosses of protonum usually red, sometimes green ; tegmina green to light green ; hind wings red, basal half blue, black-spotted ; abdomen green and yellow-green ; legs unicolorous green...*P. viridipes* Stål

    (*b*) Dark green to bright green ; bosses of pronotum dark to blackish green ; tegmina dark to bright green ; hind wings crimson-red, green at apex, unspotted ; abdomen dark green and yellow-green ; legs green, coxae and knees darker...............*P. purpurascens* Karsch

Final Instar Nymphs.

    All species are predominantly black in colour.

I.   Bosses of pronotum red or orange-red. Wing-buds yellow and black.

    (*a*) Bosses of pronotum light red or orange-red ; maculations of varying sizes, yellow ; knees purplish ; wing-buds yellow and black............
P. viridipes Stål

    (*b*) Bosses of pronotum bright red ; maculations minute, very dense and of more or less uniform size, yellow-green ; knees dull red ; wing-buds yellow-green and black...............................*P. purpurascens* Karsch

II.   Bosses of pronotum not as above. Wing-buds orange and black.

    (*a*) Bosses of pronotum and maculations (mostly large), creamy white with bright orange centres (except smallest ones), somewhat confluent on pronotum ; knees purple............*P. aegrotus* (Gerst.)

    (*b*) Bosses and maculations largely purplish so far as known ; knees purple...*P. pulcherrimus* I. Bol.

In conclusion I propose that the following standardised English names be adopted for the four species discussed :—

Phymateus aegrotus (Gerst.)—The Blue Bush Locust.

P. pulcherrimus I. Bol.—The Purple Bush Locust.

P. purpurascens Karsch—The Dark Green Bush Locust.

P. viridipes Stål—The Green Bush Locust (Green Coffee Locust).

To these might be added with advantage :—

P. leprosus (Fab.)—The Stinking Bush Locust (Stink Sprinkaan).

Summary.

The term " Bush Locust " is defined.

The synonymy and known distribution of *Phymateus aegrotus* are given and previously published records of its occurrence are reviewed.

The occurrence of hopper-bands, and of adults, in East Africa for the years 1938–47, inclusive, is outlined.

P. aegrotus and other species of the genus are normally unimportant economically as adults, but the hoppers form bands after the manner of, though on a much smaller scale than, true locusts. Outbreaks of these hopper-bands appear to be sporadic but they are sometimes destructive to crops.

P. aegrotus has usually been of only local importance as an agricultural pest but areas in which the crops have been damaged are noted. Among wild host-plants there appears to be a preference for Euphorbiaceae although the hoppers are more or less polyphagous.

Other East African species which form hopper-bands and which are known to attack crops are mentioned.

The life-history of *P. aegrotus* is described so far as it is known, and observations made by Dr. H. B. N. Hynes on the closely related species, *P. pulcherrimus*, in Ethiopia are also recorded.

Reflex actions in *Phymateus* species, including such phenomena as autohaemorrhage and colour display by the adults when alarmed, are noted and the literature on the subject is reviewed.

Observations are made on hopper behaviour and band-size of *P. aegrotus*. The latter is usually only a square yard or two in extent but bands up to 25 square yards in area have been observed by the author and there are reports of even larger ones. These bands are very dense both when marching and when stationary (60–80 final-instar hoppers per square foot). Notes on areas of infestation are also given.

In most cases it is sufficient to beat out the bands with branches. Poison bait and dusting with 7 per cent. di-nitro-ortho-cresol are also successful.

Detailed descriptions of the coloration of final-instar hoppers of *P. aegrotus* and the other East African *Phymateus* species discussed are given. Keys for the identification of these hoppers and of the adults of the four species, *P. aegrotus* (Gerst.), *P. pulcherrimus* I. Bol., *P. viridipes* Stål and *P. purpurascens* Karsch (for all of which standardised English names are proposed), are also included.

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THE DURATION OF THE AQUATIC STAGES OF *ANOPHELES MINIMUS*, THEO., DETERMINED BY A NEW METHOD.

By C. R. RIBBANDS.

Rothamsted Experimental Station.

Ribbands (1944), working in West Africa with an adult population of *Anopheles melas*, Theo., demonstrated the presence of regular fluctuations in their numbers, with peaks at intervals of eight days. These fluctuations were not readily explicable, since the evidence then available indicated that the life cycle took several days longer to complete. It is now considered, however, that the intervals between peaks were equal to the time usually taken to complete a full life cycle.

It was inferred from this work that the fluctuations in the numbers of the adult mosquito population would probably be paralleled by similar fluctuations in larval numbers. As there was no chance of studying the aquatic stages of *A. melas*, those of *A. minimus*, Theo., were studied when opportunity arose.

Collection of Data.

The experiment was conducted on the Hapjan River—a tributary of the Dibru River, at Hilika Tea Estate, Doom Dooma, Assam—one bank of which was pegged out at 1-foot intervals for a length of 100 feet. This length formed the control stretch for portions further downstream which were used for experiments on the larvicidal action of DDT (Ribbands, 1946). On 61 mornings, between 26th May and 27th July 1945, Sgt. Aspey dipped this 100-foot length, one dip per foot, and recorded the number of pupae and of larvae of each instar at each dip. All larvae were then returned to the stream. The population referred to as *A. minimus* was in reality a mixed population of group *Myzomyia*. The estimated proportions, based on identifications of 71 adults emerging from pupae, were 74 per cent. *A. minimus*, 16 per cent. *A. varuna*, Iyen., and 10 per cent. *A. aconitus*, Dön. Larvae of groups other than *Myzomyia* were separately recorded and are not now considered. The maximum and minimum water temperatures during the experiment were 33·3°C. and 19·5°C., respectively, and the average daily range was between 30·6°C. and 26·5°C. These temperatures were recorded on a thermometer which was shallowly submerged at the edge of the stream.

Method of Analysis.

Monsoon rains at irregular intervals produced great variation in the stream level, and caused very considerable variation in the width of weedy edge suitable for the Anopheline larvae. This in turn gave rise to considerable fluctuations in the average number of larvae obtained per dip, and consequently the figure obtained was not an accurate index of the number present. For this reason no attempt was made to analyse the fluctuations in terms of absolute numbers, but the irregular fluctuations caused by the storms were smoothed out by expressing the catch of larvae of each instar as a percentage of the total catch of larvae and pupae for each day.

It was hoped that the duration of the larval stages could be derived from an analysis of the correlation coefficients between the percentages of larvae of the various instars on a succession of days, but no significant or consistent results could be obtained by this method because the series of observations was too short ; very extensive series are necessary for such calculations (Kendal, 1946). Attention was then turned to the method which had been used to investigate the fluctuations in the

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C. R. RIBBANDS.

TABLE I.

Daily catches of *A. minimus* larvae and pupae.

Date	May 26	May 28	May 29	May 30	May 31	June 1	June 2	June 3	June 4	June 5	June 6	June 7	June 8	June 9	June 10	June 11	June 12	June 13	June 14	June 15	June 16	June 17	June 18	June 19	June 20	June 21	June 22	June 23	June 24	June 25	June 26	June 27	June 28	June 29	June 30
i instar	12	16	48	9	5	7	2	7	2	3	2	27	55	68	45	36	18	11	7	5	5	16	15	11	2	12	3	28	11	21	16	8	4	7	3
ii instar		7	21	9	15	22	20	29	3	6	1	6	15	20	15	11	15	20	2	5	9	14	16	32	13	10		8	13	30	22	12	14	6	10
iii instar	4	9	17	9	8	17	16	15	12	8	1	1	6		9	3	5	13	2	8	9	16	10	4	3	17	15	6		3	10	16	7	3	6
iv instar			3	5	8	16	21	3	2	8	2	3	3	2	2		1	3	3	1		4	4	1	4	5	2	10				11	5	4	5
pupae	1							4	1	6	10	2	1	2	1	3	1	1			2			3	2			5	3			1	2	1	1
Total	17	32	89	32	36	62	59	58	20	31	16	39	80	92	72	53	40	48	14	19	25	50	45	51	24	44	20	57	27	54	48	48	32	21	25

Date	July 1	July 2	July 3	July 4	July 5	July 6	July 7	July 8	July 9	July 10	July 11	July 12	July 13	July 14	July 15	July 16	July 17	July 18	July 19	July 20	July 21	July 22	July 23	July 24	July 25	July 26	July 27
i instar	6	3	7	10	6	13	18	28	17	9	1	14	24	23	8	No Record	17	14	6	6	7	1	1	2	5	18	10
ii instar	8	3	4	11	5	6	10	19	24	11	4	7	8	10	7		6	6	9	2	9	9	9	1	3	3	
iii instar	2	2		2	4	6	4	3	8	9	2	3	8	6	4		9	4	9	4	6	5	4	5	1	2	
iv instar	2	4	2	1	4	3	3	2	4	6	1	8	9	1	1		10	13	7	3	5	3	2	2			1
pupae	3		1	2		1	1	2	1	2	1	4	5	4	3		1	1		2	1	2	1	2	5		3
Total	21	12	14	26	19	29	36	54	54	37	9	36	54	44	23		43	38	32	17	28	20	17	12	14	23	14

TABLE II.

Three-day running averages, percentages of *A. minimus* of each instar.

Date	May 27	28	29	30	31	June 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
i instar	25	43	44	32	18	9	9	8	11	11	30	50	70	68	68	58	45	39	33	32	26	28	29	21	19	16	31	35	43	38	30	21	21	19	24
ii instar	45	0	24	31	35	37	40	33	28	13	13	13	18	21	21	27	34	32	28	26	30	33	42	51	48	27	12	21	39	50	42	38	33	38	36
iii instar	25	23	25	23	26	25	27	38	37	31	12	6	4	7	7	11	15	18	28	33	37	30	21	14	20	42	42	29	6	9	20	28	23	20	16
iv instar	0	0	6	13	21	28	22	17	14	17	16	8	4	2	1	2	3	10	11	9	5	6	6	9	10	13	13	9	6	0	8	13	19	18	16
pupae	3	0	0	0	0	0	2	4	10	29	29	23	3	1	3	3	3	1	1	3	3	3	2	5	5	3	3	7	7	4	1	3	4	5	8

Date	July 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
i instar	22	34	38	40	38	42	49	44	36	22	25	31	45	44	41	37	38	32	27	26	22	9	9	20	44	62
ii instar	35	31	32	32	29	25	28	36	37	39	31	28	19	23	23	22	20	19	19	24	30	43	35	27	14	0
iii instar	17	9	8	10	17	19	13	11	15	20	18	15	12	15	17	19	15	18	19	22	23	23	31	25	20	8
iv instar	21	19	17	13	12	13	7	6	9	11	16	17	14	8	10	13	20	29	27	22	17	15	15	22	18	19
pupae	6	7	5	5	4	2	3	3	4	6	9	10	10	10	9	7	4	2	5	5	9	7	11	8	6	0

adult population of *A. melas* (Ribbands, 1944), and three-day running means were used to smooth out the smaller fluctuations in the population so that the greater changes could be more readily distinguished.

Inspection showed that the peaks in the three-day running means of the various instars followed in definite sequences, and tests were applied to determine to which of several possible alternatives the sequences most closely fitted.

The Duration of the Larval and Pupal Stages.

The number of pupae and larvae of each instar caught on each day are recorded in Table I. The three-day running means of the percentages of larvae of each instar are presented in Table II, and fig. 1 is a diagram to show the distribution of the peaks in these averages. Thirty-six of the 40 peaks are very marked, and show increases of at least 20 per cent. over preceding minima, followed by decreases of at least 15 per cent. The other four peaks show increases of 10 to 20 per cent. over preceding minima.

An examination of fig. 1 indicates that the peaks are not distributed at random. There is a strong tendency for peaks of first instars to be followed within a day or two by peaks of second instars, second instar peaks to be similarly followed by those of third instars, and so on. Seven distinct sequences of peaks could be distinguished in fig. 1, and it was considered that if these sequences could be shown to be of a definite length they could only be logically explained in terms of the life history, and could therefore be used as a measure of the duration of the larval and pupal stages. Each observed sequence would be measured only from the middle of the first instar to the middle of the pupal stage, and would therefore cover approximately four-fifths of the combined larval and pupal life. Five-, six-, seven-, eight- and nine-day observed sequences would thus be approximately equivalent to a larval life of this length, or a combined larval and pupal life of 6, $7\frac{1}{2}$, 9, 10 and 11 days, respectively.
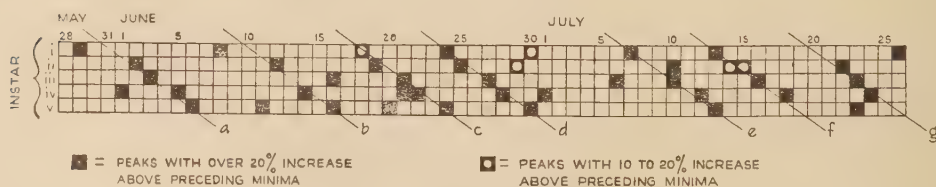
Fig. 1. Observed Sequences
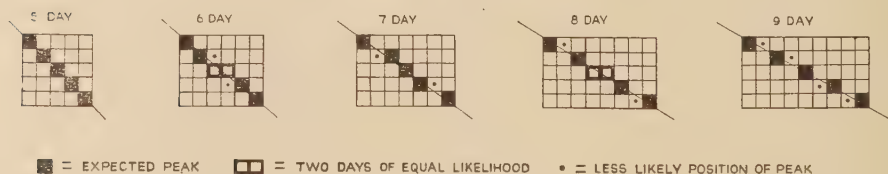
Fig. 2. Theoretical Sequences.

Proof of the existence of the peak sequences is not, of itself, of much value until it can be shown that the sequences fit a particular theoretical distribution. In fig. 2 the theoretical distribution of peaks in sequences of five-, six-, seven-, eight- and nine-day duration are shown. It will be seen that with a five-day sequence the day on which each instar should occur is quite definite, but that with six- and eight-day sequences there are two days on which the third instar is equally likely to occur, that with six-, seven- and nine-day sequences the second and fourth instars may occur on a second day in a proportion of the results, and that with eight- and nine-day sequences the first and pupal instars may occur on a second day.

The diagrams in fig. 2 are based on the supposition that the sequences begin and end on a "whole day", but it should be noted that displacement of these sequences by the maximum amount, half a day, would in each case yield a sequence which would simulate the next lower sequence in all its main peaks, and differ only in the distribution of "second preference" peaks. Therefore, when the experimenter is dealing with a true sequence for any number, he may expect that a proportion of his observed sequences will fit the next lower number slightly more accurately than the number in question.
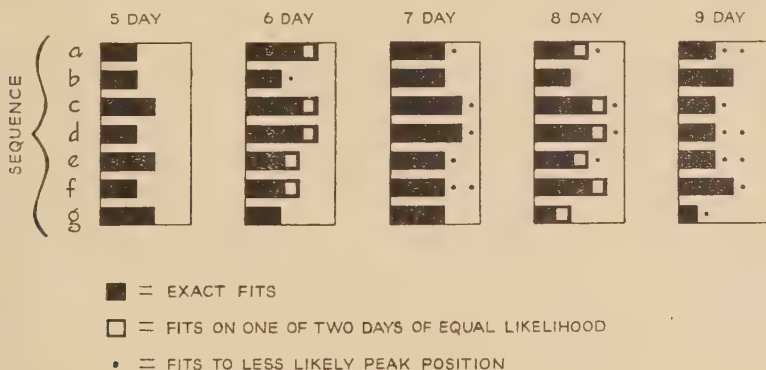
Fig. 3.—Proportion of best fits between observed and theoretical sequences.

Figure 3 represents the results of an attempt to fit each recorded sequence into each theoretical sequence. Black squares represent peaks which exactly fitted, hollow squares those which occurred on one of the two days on which they were equally likely to occur, and dots those which occurred on their day of "second preference". Figure 4 has been compiled from figure 3, and shows the distribution of best and second-best fits between the observed and theoretical sequences. From this distribution it can be seen that six out of the seven observed sequences could be fitted most closely into the theoretical seven-day sequence. In addition, the only observed sequence which fitted another theoretical sequence (the six-day one) better than the seven-day one did so by a very narrow margin, and in this instance, the seven-day sequence provided the second-best fit.
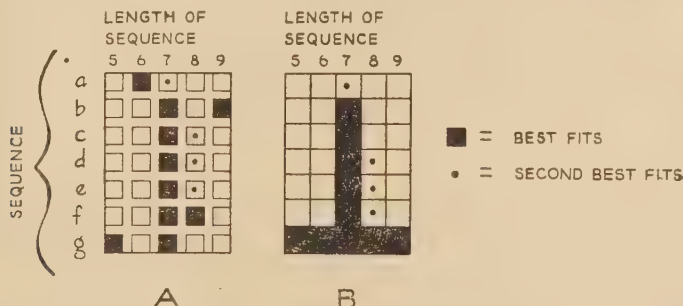
Fig. 4.—Distribution of best fits. (A) Best fits of each sequence. (B) Best fits regrouped.

It is, therefore, concluded that the larval life of the *A. minimus* under observation occupied approximately seven days, and that the combined duration of the larval and pupal life was approximately nine days.

This conclusion is in conformity with the observation that pupae of *A. minimus* were obtained from DDT-treated stretches of this stream eight days after treatment (Ribbands, 1946).

Life-Cycle of *A. minimus*.

Regular catches of adult *A. minimus* were being made in the coolie lines of the tea garden adjoining the river which was the subject of the larval observations, but unfortunately the adult catches were made primarily in connection with experiments to determine the residual effects of DDT. This programme involved daily catches in the garden, but four successive daily routines were in progress (Ribbands, 1947) ; this factor greatly intensified the difficulties of analysis, which has not been completed. In addition, the catches included *A. minimus* from many breeding places other than the Hapjan River.

However, examination of the three-day running means of the total adult catches showed that there were only three definite peaks during the time of the larval observations, and these peaks occurred on the 4th, 15th and 22nd July. Thus these adult peaks occurred four, two and three days, respectively, after the recorded pupal peaks of the fourth and fifth observed sequences and the presumed pupal peak of the sixth observed sequence, and they were followed three, five and four days later, respectively, by the recorded first instar peak of the fifth observed sequence, the presumed first instar peak of the seventh observed sequence, and the recorded first instar peak of 26th July. Thus the three aquatic sequences which were followed by observed adult peaks were also followed, exactly seven days later, by first instar peaks.

The quantity of data is insufficient to provide proof of this relationship, but this evidence is regarded as suggestive, and it is considered that it indicates that under these conditions the complete life cycle occupied a period of 14 days, of which the egg stage occupied two days (Thomson, 1940), the larval life seven days, and the combined larval and pupal life approximately nine days, leaving three days for the period between adult emergence and egg-laying.

This is a shorter period than that hitherto accepted for the life cycle of *A. minimus*, but the divergence can be wholly explained in terms of the duration of larval life, which very careful estimates had indicated to occupy 9 to 12 days (Thomson, *ibid.*). These estimates were based on experiments conducted in floating cages, in a typical breeding place with a water temperature range from 34·0°C. to 27·0°C. The water temperature in the Hapjan River was slightly lower than this, yet larval development occurred in only seven days. It is thought that this indicates that caged larvae are exposed to conditions less favourable than those which occur in nature (probably because in nature they can change their environment more frequently, or insert themselves into small crannies which contain very rich food supplies), and that these less favourable conditions tend to delay their development.

Summary.

A new method of determining the duration of the aquatic stages of Anophelines in nature is described. Repeated daily dippings of a stretch of stream were made, and the numbers of pupae and of larvae of each instar were recorded. The duration of the aquatic stages was then determined by analysis of the sequences of peaks in these numbers.

The duration of larval life of *A. minimus*, when living in a stream at an average daily range of water temperature between 26·5°C. and 30·6°C., was seven days.

The full life cycle of *A. minimus*, under monsoon conditions in Assam, probably occupied 14 days—egg 2 days, larva 7 days, pupa 2 days, adult to first egg-laying 3 days.

Acknowledgements.

Sgt. J. A. Aspey, R.A.M.C., of No. 2 Entomological Field Unit, was responsible for the whole of the field work and the results are testimony to his accuracy and reliability, while working under very unpleasant monsoon conditions.

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EXPERIMENTAL AERIAL SPRAYING WITH DDT AGAINST MOSQUITOS IN BURMA.

By T. W. Tyssul Jones, M.Sc.

(Plate VI.)

A considerable amount of work has been carried out in Indian areas, West Africa and elsewhere on the application of a DDT solution from the air. Although there is unanimity of opinion regarding optimum conditions for the actual operation of spraying from aircraft, there is a great deal of controversy concerning the actual effectiveness of the method against mosquito adults and larvae. It is agreed that a complete kill of larvae breeding in water open to the sky is obtained, but many of the trials, although carried out in the field, were experimental ; saucers or other flat containers were used and the effect on the larvae contained therein accepted as a measure of the effect of DDT solution on larvae breeding in the open. In other cases open pools with no vegetative growth were sprayed with the same result.

In the case of larvae breeding in pools under cover of overhanging trees, very inconsistent results were obtained, satisfactory kills and the reverse being recorded from areas but a few yards distant. This is ascribed to the filtration effect of the vegetation on the spray.

The present contribution is the result of an investigation carried out by the author on the effect of aerial spraying of DDT solution against mosquitos on Akyab Island, Burma.

An attempt was made to determine whether DDT solution applied from the air has any action on larvae breeding in open ponds containing varying degrees of vegetative growth. Also whether the filtration of the spray by closely growing tall trees protects the shrubs at ground level from contamination and so provides safe shelter for resting adult mosquitos.

With these ends in view a thorough survey of the area to be sprayed was carried out and it was finally decided to study Royal Lake for the action on larvae and Thetkebyin village for the effect on adults.

Description of the Areas.

Royal Lake.

This lake offered several advantages in that it was open, with no high trees in the vicinity to impede the falling spray and that it had a fairly high and constant larval population. It is approximately a mile by half a mile in surface area. Its longer axis runs north and south and from the northern bank, there projects a tongue of land (fig. 1) with a long and wide base over which is scattered a number of pools surrounded by bunds. These pools and the general surface of the lake along this northern bank are covered with different types of vegetation ; in certain sections it was sparse, in others it consisted of the water plant, *Limnanthemum*, while a few of the pools contained heavy growths of a brown alga. Ten test points extending for about five hundred yards along this bank were selected in such a way that, as far as possible, areas of sparse vegetation alternated with the other different types. These test points are listed below.

Points 1, 4, 6, 8, 9 and 10 were areas with sparse vegetation (Plate VI, fig. 1). They had a very narrow border of grass the blades of which projected 9 to 12 inches

above the water surface but so widely distributed that the water was quite visible between.

Point 2 consisted of a 4- to 5-yard wide border completely covered with the water plant, *Limnanthemum* (Plate VI, fig. 2). This area extended for approximately 10 yards.

Area No. 3 was partially covered with *Limnanthemum* and extended for about 4 to 5 yards; much of the water surface was visible between the leaves. Unfortunately this is not illustrated by photograph.

Point 5 was situated on the base of the tongue of land and consisted of a rectangular pool 10 yards long by 5 yards wide. There was a one-yard border of thick grass, so thick that the general surface of the water was quite invisible (Plate VI, fig. 3).

Point No. 7, a pool, was again situated on the base of the tongue of land and had the same dimensions as the preceding pool. There was a one-foot-wide border of thick grass which projected about 12 inches above the water surface. The general surface of the pool was covered with a brown algal growth (Plate VI, fig. 4).
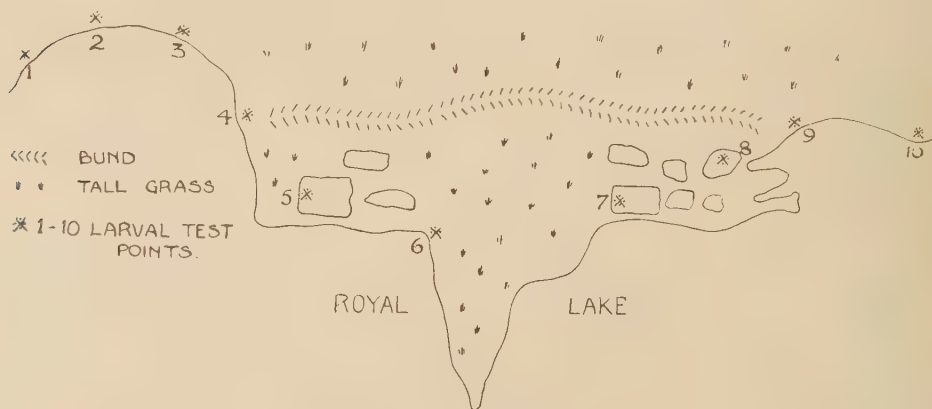
SKETCH OF PART OF ROYAL LAKE SHOWING DISTRIBUTION OF THE LARVAL TEST POINTS.

Fig. 1.

Thetkebyin Village.

This village at present co sists of Old Th tke' yin and Thetkebyin Block, the two being separated by a belt of paddy fields about a mile wide. They are markedly different in topographical features. The older part of the village, Old Thetkebyin, is a characteristically straggling village consisting of scattered huts in clearings of dense woodland. The paths connecting the various native dwellings pass through thick scrub. This section, therefore, has areas of dense shade at ground level inter-mingled with areas of partial shade in the less wooded parts and light conditions in the clearings. In marked contrast, Thetkebyin Block is composed of a compact mass of about 40 recently constructed huts, all on open ground. At the time of this investigation, prior to the monsoon rains, the paddy fields were dry, the only water collections present being the wells from which the natives drew their meagre domestic water supply and a few ponds, the water in which was highly polluted with the excreta of animals.

The huts were of two distinct types.

Huts with corrugated iron roofs (Plate VI, fig. 5).

These were only found in Old Thetkebyin. The roof, front and back walls were of corrugated iron sheets with a considerable space between the roof and the walls. The sides were of bamboo. Relatively few had a door and the interior of all was quite light. Some of these huts were in the open, others in dense or partial shade.

Huts with thatched roofs (Plate VI, fig. 6).

All the huts in Thetkebyin Block and some in Old Thetkebyin were made completely of bamboo. The upper third of the side walls were of open lattice work but were more or less protected by the roof over-hang. There was a considerable space between both the front and back walls and the roof.

Conditions prior to Spraying.

Royal Lake.

There are certain species of mosquito larvae which are very sensitive to external stimuli. The shadow cast by the dipper or the mere touch of the water will immediately send them scurrying to the bottom. In order to determine the species breeding in the lake, 150 specimens were collected and identified. Three species were found, *Anopheles jamesi*, Theo., *A. annularis*, Wulp, and *A. hyrcanus*, Pall., and as these species are not sensitive to the above stimuli, the method of dipping adopted gave an accurate representation of the larvae present during the investigation.

The dipper used was an enamel dish 12 ins. by 9 ins. and 4 ins. deep. This was dipped into the lake and water allowed to flow in until it was half full. The larvae were counted by pipetting out into a specimen tube.

Dips at all the test points were taken on each of five days prior to treatment in order to ascertain the density and possible fluctuation of the larval population. The larvae were put back into the lake in each case after the number had been noted. The results are given in Table I.

TABLE I.

Date and Time	Points									
	1	2	3	4	5	6	7	8	9	10
5th April, 10.30 ...	6	21	14	2	16	0	14	10	4	3
6th April, 10.30 ...	8	22	18	2	17	1	12	9	2	3
7th April, 10.30 ...	4	19	14	1	14	2	10	7	3	2
8th April, 10.30 ...	5	19	16	2	13	1	13	8	4	3
9th April, 10.30 ...	5	20	15	1	15	0	15	9	3	2

1. Sparse vegetation.   2. Thick *Limnanthemum*.   3. Sparse *Limnanthemum*.
4. Sparse vegetation.   5. Wide border of thick grass.   6. Sparse vegetation.
7. Pool with alga.   8–10. Sparse vegetation.

Thetkebyin.

A. vagus, Dön., was the only Anopheline species found in the area. This was unfortunate as it was hoped to encounter mosquitos that rest out of doors by day and enter houses only for the purpose of feeding on the one hand and those that feed and rest indoors and leave only for oviposition on the other. Nevertheless, the habits of *A. vagus* were admirably suited to the investigation because it is essentially a cattle feeder. It was noted during night catches previous to the spraying that *A. vagus* was caught in the huts about dusk but very few after that until about dawn; they leave the huts to feed on cattle but rest out of doors for a considerable period before returning to their daytime resting places in the huts.

In order to determine the density of population, six huts were examined at dusk and at hourly intervals until dawn for a number of nights prior to the spraying. The figures in Table II are the averages for the six huts.

TABLE II.

Old Thetkebyin		Thetkebyin Block	
5th/6th April ...	12	12th/13th April ...	4
7th/8th April ...	6	13th/14th April ...	6
9th/10th April ...	14	15th/16th April ...	5
11th/12th April ...	18	20th/21st April ...	4
18th/19th April ...	12		
22nd/23rd April ...	22		

Effect of Aerial Spraying.

Estimation of the concentration in milligrammes per square metre.

The absence of the necessary equipment made it impossible to estimate the concentration of spray reaching the ground with great accuracy, but the method employed appears to give a reasonably accurate figure.

Test envelopes were placed in various positions. After the spray had been allowed to settle, the spots were kept under observation to ascertain whether there was any increase in size.

A similar test envelope was then taken and by means of a graduated one millilitre pipette, 0·05 ml. 5 per cent. DDT solution (as used in the field spraying) was spotted on to it, the size of the spots being made to agree as nearly as possible with those on the field test papers. The diameter of each spot was measured by means of a knife-edged vernier and the total area of the spots calculated. The average of a number of such tests gave the total area carrying the known weight of DDT deposited by 0·05 ml. 5 per cent. DDT solution.

By measuring the areas of the spots on the field test papers and comparing the total area thus obtained with that carrying the known weight of DDT, the total number of milligrammes per square metre was calculated.

Wind speed and direction.

The Meteorological Department supplied the following data as those applying at the times of spraying the Royal Lake area and the Thetkebyin area. At the Royal Lake, at 09.00 and 10.00 hours, the wind speed was 3 and the wind direction E.N.E. The same conditions prevailed at Thetkebyin at 09.00 hours, but at 10.00 hours the wind speed was 2 and the wind direction N.N.W.

The wind speed on both occasions was negligible and the spray fell more or less vertically with little lateral drift.

Effect of aerial spraying against larvae.

All the test papers placed along the northern bank of the lake showed a concentration of DDT ranging from two to three mg. per square metre. The resultant effect of the spray is given in Table III which shows the number of larvae taken at the various points over a period of ten days.

A number of interesting points emerge from an examination of this table. In the first place, even before spraying, larvae were much less numerous in areas of sparse vegetation than in areas with thick growth. A possible explanation for this is the fact that microscopic growth is much more abundant amid vegetation than

TABLE III.

Date and Time	Points									
	1	2	3	4	5	6	7	8	9	10
Day before spray ...	5	20	15	1	15	0	15	9	3	2
Day of spray										
10th April, 10.30 ...	2	14	5	0	4	0	6	8	2	1
10th April, 16.00 ...	0	15	0	0	7	0	4	0	0	0
11th April, 10.30 ...	0	12	0	0	4	0	3	0	0	0
14th April, 10.30 ...	0	14	0	0	4	0	3	0	0	0
20th April, 10.30 ...	0	12	2	0	5	0	5	0	0	0

1. Sparse vegetation. 2. Thick *Limnanthemum*. 3. Sparse *Limnanthemum*.
4. Sparse vegetation. 5. Wide border of thick grass. 6. Sparse vegetation.
7. Pool with alga. 8–10. Sparse vegetation.

it is in open water. It may be, however, that the growing plant, with its accompanying debris, offers protection and shelter from larval predators. The negative or positive heliotropism exhibited by certain species does not apply here as the three species concerned occur equally in both sunlit and shady conditions. In areas of sparse vegetation the larvae were killed in six hours.

The area of water completely covered with *Limnanthemum* (Point 2), although showing an initial fall from 20 to 14, remained fairly constant afterwards. Test point number 3 with a slight covering of the water plant showed a complete kill by 16.00 hours on the day of treatment. On 20th April, two, and on subsequent days more, third and fourth instars were collected from this area, yet on 14th April there had been none. These two larvae cannot have been the result of fresh hatching as they could not have reached the third instar in six days. It is thought that they must have migrated from the area of thick *Limnanthemum* growth as the areas are adjacent. Their presence in this area suggests that the effectiveness of DDT on water does not last longer than six days.

The pool with a very wide border of thick grass showed a considerable drop from 15 to 4, at which figure it remained fairly constant. The same applies to the pool covered with algal growth.

Even after 10 days, larvae had not reappeared in the areas of sparse vegetation. This, however, was not due to any residual effect of the DDT but possibly to the marked preference of larvae for the more thickly vegetated water surfaces and the diminution of adult Anophelines in the neighbourhood as a direct effect of the spray.

Effect of aerial spraying against adults.

The effectiveness of DDT solution against adult Anophelines depends essentially upon the spray contaminating the resting places of the mosquitos. A large number of test papers were placed on ground open to the sky and on ground in partial and in dense shade to determine whether heavily wooded areas have a filtrating effect on the falling spray. The concentration of DDT in milligrammes per square metre under these conditions was as follows :—

Open ground	*Partial shade*	*Dense shade*
2·0–3·0	0·5–0·7	Trace

Trace represents less than 0·05 mg. per square metre.

It is obvious that in heavily wooded areas the spray is prevented from penetrating to ground level, at least under the conditions prevailing. Small shrubs in dense shade, which provide ideal resting places for adult Anophelines, are therefore more or less uncontaminated by the spray. Shrubs in the open have a relatively high concentration, whereas those in partial shade have a concentration varying between 0·5 and 0·7 mg. per square metre.

A number of night catches was carried out in Old Thetkebyin and Thetkebyin Block. The results are given in Table IV.

<div align="center">TABLE IV.</div>

Old Thetkebyin		Thetkebyin Block	
Date	No. of Anophelines	Date	No. of Anophelines
Day before Spraying	22	Day before Spraying	10
23rd/24th April ...	3	23rd/24th April ...	Nil
24th/25th April ...	4	26th/27th April ...	,,
26th/27th April ...	4	28th/29th April ...	,,

The above figures represent the average for six huts in each case.

In the case of Thetkebyin Block there was a complete kill. This is attributed to the fact that the village was open, with no high heavily wooded areas protecting the shrubs which offered a resting place for the mosquitos after they had fed upon the cattle.

There was a considerable drop in Old Thetkebyin from 22 to 3 or 4 Anophelines, but the kill was not complete. The number remained more or less constant until 28th April and was no doubt due to the fact that the shrubs in heavy cover offered safe resting places for the adults. The area was sprayed on the 28th by a unit using hand pumps and the following night the catches were all negative.

Penetration of spray into huts.

The penetrative power of aerially sprayed DDT solution into huts and other dwellings is of fundamental importance when the object is to control a disease carried by vectors which rest indoors. A number of huts of the types described above was selected in order to determine this penetrative power ; some of these were in open ground within a few yards of test papers showing a concentration of 2–3 mg. per square metre, whilst others were in different shade conditions. Eight test papers were placed in each, but no trace of DDT was found on them after the spray had settled. Hence under the conditions prevailing at the time of the spraying there was no penetration of aerially sprayed DDT into huts of the type described.

Discussion.

It would seem that in ponds having an Anopheline population, vegetative growth offers protection against aerially sprayed DDT solution, the degree of protection depending upon the type of the vegetation and the concentration of the solution falling upon such water surfaces. With concentrations of 2 to 3 mg. per square metre, the water plant, *Limnanthemum,* gives complete protection if the growth is such that the leaves cover the water surface, but only partial protection if there is a considerable surface of water open to the effect of the spray. It is obvious also that tall thick grasses must have a considerable filtrating effect, the extent of which will depend upon the angle at which the spray hits the blades. During the present investigation the spray, owing to negligible wind, fell more or less vertically, yet there was a partial protection. It is suggested that with higher wind velocities, causing the spray to strike the blades at a more oblique angle, the filtration effect will be enhanced.

The most important property of DDT as a larvicide or insecticide is its residual action. In this investigation the residual effect against mosquito larvae breeding in water has been far from convincing. Six days after a complete kill had been

achieved, larvae were found breeding in water which had been subjected to con-
centrations of 2 to 3 mg. per square metre.   Experiments carried out in India with
much higher concentrations, even under laboratory conditions, showed that the
residual effect on water lasts no longer than 9 to 10 days.

The extent to which an air spray penetrates vegetation is an important factor in
the control of both mosquito larvae and adults.   It has been shown above that
vegetative growth of different kinds growing in ponds open to the sky give protection
to mosquito larvae against an aerially sprayed DDT solution.   It is obvious therefore
that if such ponds are protected by a canopy of trees the filtration effect will be
much increased, thus affording greater protection.   In the present investigation it
was seen that the filtration effect was severe, areas in dense shade only showing a
trace of DDT.   Shrubs growing at ground level in heavily shaded areas were more or
less protected from the spray, under the conditions of concentration and wind
prevailing at the time.   It is suggested that an increase in wind velocity will not
cause a great deal of difference in the penetration except perhaps on the outside
edges of heavily wooded areas.   Much greater amounts of spray may penetrate
farther, but it is not believed that the results would be commensurate with the
cost.   In open scrub land, aerially sprayed DDT solution can give a 100 per cent.
kill even at low concentrations.

Under conditions of negligible wind there is no penetration into huts of the type
shown in the figures, although some of them were within a few yards of test points
showing 2 to 3 mg. per square metre.   It is doubtful whether wind velocities of
much greater strength, but practicable for aerial spraying, would cause sufficient
penetration to produce a residual effect.

Summary.

The effect of aerially sprayed 5 per cent. DDT solution against Anopheline larvae
and adults has been investigated.

An account is given of a rapid field method for estimating the concentration in
milligrammes per square metre.

At a concentration of 2–3 mg. per square metre, aerially sprayed DDT solution
had little effect on Anopheline larvae breeding in water completely covered with a
species of *Limnanthemum*.   Also at this concentration tall grass gave partial
protection, whereas in open water there was a complete kill.

The residual effect of DDT at a concentration of 2–3 mg. per square metre did
not last longer than six days.

Under negligible wind conditions, resulting in a vertically falling spray, there was
no penetration into huts even when situated in the open.

Drastic filtration of the spray resulted in a fall in the concentration from 2 to 3
mg. per square metre in the open to less than ·05 mg. per square metre under heavy
shade, consequently shrubs at ground level in heavy wooded areas were greatly
protected from contamination with DDT and afforded practically safe resting places
for adults.

PLATE VI.

Fig. 1. Area with sparse vegetation. Test points Nos. 1, 4, 6, 9 and 10.

Fig. 2. Area completely covered with a plant, *Limnanthemum*. Test point No. 2.

Fig. 3. Pool with wide border of thick grass. Test point No. 5.

Fig. 4. Pool with narrow border of thick grass, the remaining water surface covered with a brown algal growth. Test point No. 7.

Fig. 5. Hut with roof, front and back composed of corrugated iron, the sides of bamboo.

Fig. 6. Hut made completely of bamboo.

STUDIES ON WEST AFRICAN FOREST MOSQUITOS.
PART II. THE LESS COMMONLY OCCURRING SPECIES.

By P. F. MATTINGLY.

(From the *Yellow Fever Research Institute, Yaba, Lagos, Nigeria*.)*

The details of the area studied and of the techniques employed have already been given elsewhere (Mattingly, 1949) and will be repeated here only in outline. The Field Station, consisting of a single tree fitted with four platforms, at ground level, 22 ft., 40 ft. and 52 ft., respectively, was situated on the left bank of the Ogun River about three miles from the point at which it flows into Lagos Lagoon, S. Nigeria. Each platform was designed to hold two Africans whose duty it was to catch mosquitos on themselves and on one another. Each individual catch was continued for 24 hours without a break, during which time the boys were kept under constant supervision. Every two hours the boys were changed from one platform to another in order so far as possible to eliminate artificial periodicities due to variations in individual attractiveness to mosquitos or other causes. The mosquitos were collected punctually at the end of each hour and at the same time hourly psychrometer readings were taken. The biting cycle of each species, as defined later, was determined by summing the total numbers of that species biting during each hour over a series of 22 catches. The figures were broken down by platforms to obtain vertical distribution data and, to secure a picture of the seasonal variation in numbers, the monthly totals were divided by the number of catches made in each particular month.

The Mosquito Population.

The four species *Taeniorhynchus africanus*, Theo., *Anopheles hargreavesi*, Theo., *Anopheles gambiae*, Giles, and *Aëdes africanus*, Theo., have already been discussed in detail (Mattingly, 1949) and will be referred to in the present paper only incidentally. Of the remaining species, which are listed below, only the first ten were sufficiently abundant to afford a reliable indication of their biting cycles and vertical distribution. The full list of species taken was as follows :—

Anopheles (A.) paludis, Theo.
Anopheles (Myzomyia) moucheti var. *nigeriensis*, Ev.
Aëdomyia africana, N.-L.
Aëdes (Mucidus) grahami, Theo.
Aëdes (Aëdimorphus) nigricephalus, Theo.
Aëdes (Aëdimorphus) irritans, Theo.
Aëdes (Banksinella) circumluteolus, Theo.
Aëdes (Diceromyia) flavicollis, Edw.
Aëdes (Diceromyia) taylori, Edw.
Culex (Culex) thalassius, Theo.
Anopheles (A.) coustani var. *ziemanni*, Grünb.
Anopheles (A.) obscurus, Grünb.
Anopheles (Myzomyia) nili, Theo.
Anopheles (Myzomyia) pharoensis, Theo.
Uranotaenia pallidocephala, Theo.
Uranotaenia philonuxia, Edw.
Uranotaenia annulata, Theo.
Uranotaenia ornata, Theo.
Ficalbia (Mimomyia) hispida, Theo.
Ficalbia (Mimomyia) mimomyiaformis, Newst.
Ficalbia (F.) uniformis var. *malfeyti*, Newst.
Taeniorhynchus (Coquillettidia) annetti, Theo.
Taeniorhynchus (Coquillettidia) aurites, Theo.
Taeniorhynchus (Mansonioides) uniformis, Theo.
Aëdes (Finlaya) longipalpis, Grünb.

Aëdes (Finlaya) ingrami, Edw.
Aëdes (Stegomyia) apicoargenteus, Theo.
Aëdes (Aëdimorphus) punctothoracis, Theo.
Aëdes (Aëdimorphus) domesticus, Theo.
Aëdes (Aëdimorphus) albocephalus, Theo.
Aëdes (Banksinella) punctocostalis, Theo.
Culex (Neoculex) andreanus, Edw.
Culex (Neoculex) rima, Theo.
Culex (Neoculex) subrima, Edw.
Culex (Neoculex) insignis, Cart.
Culex (Neoculex) sunyaniensis, Edw.
Culex (Neoculex) wigglesworthi, Edw.
Culex (Mochthogenes) inconspicuosus, Theo.
Culex (C.) poicilipes, Theo.
Culex (C.) decens, Theo.
Culex (C.) perfuscus, Edw.
Culex (C.) perfidiosus, Edw.
Culex (C.) guiarti, Bl.
Culex (C.) ingrami, Edw.
Culex (C.) philipi, Edw.
Taeniorhynchus (Coquillettidia) metallicus, Theo. (males only).
Ficalbia (Mimomyia) pallida, Edw. (male only).
Aëdes (Aëdimorphus) tarsalis, Newst. (male only).

E

A number of specimens of *Anopheles obscurus* was of the form described as
"var. *nowlini*" by Evans (1932) but stated by De Meillon (1947) to vary in the
characters originally regarded as diagnostic. The specimens of *Aëdes grahami*
corresponded in general with the pale form described by Edwards (1941) but had
many pale scales on the costa and a variable number of pale scales in the dark area
on the front tibia.

Aëdes taylori, as recorded by Lewis (1945) from the Sudan, had numerous
scattered pale scales on the dorsal surface of the abdomen and their identity was
established by examining the terminalia of the males by which they were accom-
panied. The members of the *Culex rima* group (*Culex rima*, *subrima*, *insignis*,
sunyaniensis and *wigglesworthi*) were also separated by the characters of the male
terminalia and it was not found possible to identify the females with confidence. The
same was true of *Culex perfuscus* and *C. perfidiosus*. All the available data regarding
the less abundant species are summarised in Table I which shows the numbers taken
and the date, time and platform on which or at which they occurred. All times
given throughout the present paper are Local Mean Time and represent the times
at which the mosquitos were collected from the platforms, i.e., the ends of the one-
hourly periods during which they were taken into the collecting tubes by the boys.
The platforms are numbered in ascending order from the ground upwards, i.e., the
ground platform is numbered I, the 22 ft. platform II, the 40 ft. platform III and
the top platform at 52 ft. IV.

Males.

Except where otherwise stated, all remarks made and figures given in the present
paper refer to female mosquitos only. Males of a number of species were, however,
taken and in three species, *Taeniorhynchus africanus*, *Aëdes flavicollis* and *A. taylori*,
they were abundant. Table II shows the vertical distribution of males of these
three species and the proportions of the total catch occurring during each hour. It
will be noted that in each case the proportion of the total catch of males taken during
the hour after sunset (hour ending 19·15) is very much greater than in the case of the
females. The vertical distribution of the two sexes is also somewhat different.
Thus, the majority of the females of *Taeniorhynchus africanus* occur on the ground
whilst the males occur in greatest abundance on the two middle platforms. As
against this, however, the proportion of females occurring on the middle platforms
is very much greater during the two hours following sunset than at other times during
the night (Mattingly, 1949). The great concentration of both males and females
on the middle platforms during this period strongly suggests a swarming activity
associated with mating. In the case of *Aëdes flavicollis* and *A. taylori*, which are
predominantly tree-top biters, the vertical distribution of the females is not very
markedly different during the early hours of darkness from the remainder of the
night. All that can be said is that, in general, it agrees fairly closely with that of the
males and so is not at variance with a hypothesis which postulates the occurrence of
mating or other activities associated with swarming during the early part of the
night (Table VI).

Other species of which males were taken are given in Table III.

Rainfall and seasonal Distribution.

The seasonal distribution curves shown in fig. 1 are based on average monthly
catches, i.e. on the monthly totals divided, in each case, by the number of catches
made during the month in question. The figures on which the curves are based are
given in Table IV. The rainfall curve superimposed for purposes of comparison on
the distribution curves is based on figures given in Mattingly (1949). No catches
were made during December 1945 and January 1946. The dates given in Table IV
refer to the days on which the catches were begun. Since each catch was begun at

TABLE I.

Data relating to the less abundant species at Itowolo.

Species	Monthly Catches 1945 Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	1946 Feb.	Mar.	Apr.	May	Jun.	Platforms I	II	III	IV	4-hourly Periods* 1	2	3	4	5	6	Total Catch
An. coustani	1	1	2	—	1	6	—	—	—	—	3	5	4	1	2	—	7	3	1	1	—	12
An. obscurus	—	2	1	—	3	1	—	—	—	—	—	10	—	1	1	5	2	2	1	1	—	11
An. nili	—	1	—	6	10	—	—	—	1	—	—	7	3	8	—	1	1	3	5	1	—	11
An. pharoensis	4	2	6	7	6	7	—	1	1	—	1	16	9	8	2	—	7	11	16	1	—	35
U. pallidocephala	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
U. philomaxia	—	4	1	5	1	—	—	—	—	—	—	3	1	2	4	—	7	3	—	—	—	10
U. annulata	—	1	1	1	1	—	1	—	—	—	—	1	1	1	1	—	1	—	2	—	—	3
U. ornata	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	—	—	—	3
F. hispida	—	—	—	—	—	—	—	—	—	—	1	—	—	1	1	—	1	1	—	—	—	1
F. mimomyiaformis	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	1
F. uniformis	—	9	—	1	1	—	—	—	—	—	—	—	10	10	6	2	14	8	4	—	—	3
T. annettii	15	—	—	1	1	—	—	—	—	—	1	—	10	10	1	1	1	8	—	2	—	26
T. aurites	—	4	7	1	1	—	—	—	—	—	—	8	7	3	6	—	3	9	10	2	2	3
T. uniformis	1	—	2	5	—	—	—	—	—	—	1	—	2	—	—	—	—	—	—	—	1	24
Aë. longipalpis	—	3	4	—	—	—	—	—	—	—	—	1	3	—	1	2	—	—	3	3	2	2
Aë. ingrami	1	1	—	—	—	—	—	—	—	—	—	3	—	—	—	1	—	—	—	—	1	1
Aë. apicoargenteus	—	3	4	2	—	—	—	—	—	—	—	15	—	—	—	7	2	—	—	—	—	7
Aë. punctothoracis	7	5	—	2	—	—	1	—	—	—	—	3	—	—	1	1	2	1	4	4	2	1
Aë. domesticus	1	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—	1	1	2	2	3	15
Aë. albocephalus	—	—	4	2	—	8	1	—	—	—	—	—	1	1	—	—	—	2	—	2	—	3
Aë. punctocostalis	—	2	6	1	1	—	—	—	—	—	—	6	2	2	5	2	5	2	1	2	2	6
C. andreanus	1	5	1	2	3	—	—	—	—	—	—	1	1	2	4	1	2	1	2	—	—	1
C. rima group	1	1	2	1	—	2	—	—	—	—	1	1	1	2	11	—	5	4	5	—	—	15
C. inconspicuosus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	2	2	1	—	—	4
C. poicilipes	6	2	2	1	54	—	3	—	—	—	26	19	13	20	19	28	18	10	—	3	15	15
C. decens	13	—	—	—	5	—	1	—	1	—	—	—	13	5	—	—	4	1	—	—	4
C. perfuscus group	—	—	—	2	—	3	—	—	—	—	1	1	1	—	—	3	3	1	—	—	3	78
C. guiarti	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
C. ingrami	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
C. philipi	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1

*The respective 4-hourly periods are numbered as follows: 1.—14.15–18.15.   2.—18.15–22.15.   3.—22.15–02.15.   4.—02.15–06.15.   5.—06.15–10.15.   6.—10.15–14.15.

E2

TABLE II.

Distribution of male mosquitos at Itowolo.

Species	Platform	16.15	17.15	18.15	19.15	20.15	21.15	22.15	23.15	00.15	01.15	02.15	03.15	04.15	05.15	06.15	07.15	
								Time (L.M.T.)										
T. africanus	IV	—	—	—	4	3	1	0	4	2	2	1	5	4	2	1	—	29
	III	—	—	1	23	19	10	8	17	8	10	12	10	3	3	2	—	125
	II	—	1	4	45	30	14	13	19	13	10	12	18	8	13	1	—	198
	I	1	2	5	5	14	14	3	8	8	9	1	10	9	8	2	—	97
	Total	1	2	5	77	66	39	24	48	31	31	26	43	24	26	6	—	449*
% of Total Catch ...		0·2	0·4	1·1	17·1	14·7	8·7	5·3	10·7	6·9	6·9	5·8	9·6	5·3	5·8	1·3	0·0	99·8%
Aë. flavicollis	IV	—	—	2	73	23	7	4	5	5	5	6	7	6	3	—	—	146
	III	—	—	6	75	15	2	5	5	5	3	4	3	2	1	—	—	126
	II	—	—	1	10	10	2	—	1	—	1	2	—	1	—	—	—	28
	I	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
	Total	—	—	9	159	48	11	9	11	10	9	12	10	9	4	—	—	301
% of Total Catch ...		0·0	0·0	3·0	52·8	15·9	3·7	3·0	3·7	3·3	3·0	4·0	3·3	3·0	1·3	0·0	0·0	100·0%
Aë. taylori	IV	—	—	3	41	15	2	4	7	4	1	—	1	1	1	—	1	79
	III	—	—	2	40	13	2	3	1	2	1	—	—	2	1	—	—	67
	II	—	—	—	12	7	2	—	—	—	—	—	—	1	—	—	—	24
	I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
	Total	—	—	5	93	35	6	7	8	6	2	—	1	4	2	—	1	170
% of Total Catch ...		0·0	0·0	2·9	54·7	20·6	3·5	4·1	4·7	3·5	1·2	0·0	0·6	2·4	1·2	0·0	0·6	100·0%

*1 male of *T. africanus* was taken on platform I during the hour ending 15.15. No males of *A. flavicollis* or *A. taylori* were taken after 07.15.

TABLE III.—Distribution of Males. Except where indicated by figures in brackets, only one male was taken.

Species	15.15	16.15	17.15	18.15	19.15	20.15	21.15	22.15	23.15	0.15	1.15	2.15	3.15	4.15	5.15	6.15	7.15	8.15	9.15	10.15	11.15	12.15	13.15	14.15
Aë. africanus					IV					III														
Aë. albocephalus							IV		IV															
Aë. domesticus						IV																		
Aë. nigricephalus					IV	IV			I													I	I	
Aë. tarsalis										IV														
An. gambiae	I																							
An. paludis											IV			IV										
Ur. philonuxia									II															
Ur. annulata											IV				III									
Aëdo. africana					III & IV	II & IV																		
Fic. pallida								III																
Fic. malfeyti						IV	III									III								
T. annetti				IV		IV																		
T. metallicus														IV	IV						I			
Cu. insignis		I			III & IV		ALL	IV		IV	IV		IV											
Cu. rima					II	III	II	II (3)	IV (2)	II, IV														
Cu. subrima						IV	I		I	III														
Cu. sunyaniensis						IV																		
Cu. inconspicuosus					IV	IV	IV																	
Cu. perfuscus					I & II	II (3), III, IV	III (2)		I, II, III	II, IV		IV		IV										
Cu. perfidiosus					IV	IV	III	IV																
Cu. ingrami										IV	IV													
Cu. philipi									III															

Fig. 1.—Seasonal distribution at Itowolo. (Rainfall indicated by broken line.)

15.15 hours on one day and continued until 15.15 hours on the next, each catch covered two successive days. The curves for *Aëdes flavicollis* and *A. taylori*, the only two tree-hole breeders shown, should be compared with that for *A. africanus* (Mattingly, 1949), also a tree-hole breeder. The resemblance is very close. It will be noted that the species in question are most abundant before the occurrence of maximum rainfall in July, although in all the other species the period of abundance follows the period of maximum rainfall. As against this a small secondary peak occurs at the end of October and follows the " small rains". It appears likely from this that the optimum rainfall for these species is well below the maximum which actually occurs and that, whereas sufficient rain to fill the tree-holes is required, too much will wash them out. Alternatively if a long breeding cycle is envisaged it may be that the peak rainfall in July coincides with a period of exhaustion of the breeding-places following on the initial stimulus provided by the onset of the rains in April. *Aëdes irritans*, a crab-hole breeder, shows a similar curve and here the same arguments would apply. *Anopheles paludis* shows a very marked dry season peak and resembles in this respect *A. hargreavesi* and *Taeniorhynchus africanus* (Mattingly, 1949), a fact which probably indicates a similarity in breeding-places, but *Aëdomyia africana*, which also breeds in *Pistia*, has quite a different type of curve. The accumulation of large masses of *Pistia* in the creeks around Itowolo is bound up with their closure and isolation from the river during the dry season. Heavy flood water tends to sluice them out. *Aëdes grahami*, a ground-pool breeder with a cannibalistic larva, reaches its peak well after *Anopheles gambiae*, and the reduction in numbers of the latter species after the middle of August may be accounted for in part by the presence of larvae of *A. grahami* in its breeding-places.

Other meteorological and microclimatic data are given in Mattingly (1949).

Biting Cycles.

A discussion of the precise implications of the term " biting cycle " will be found at the end of the present paper. For practical purposes it may be taken to imply the hour to hour changes in the numbers of any particular species taken during the course of a standard twenty-four hour catch. Where, as in the present instance, a number of such catches are made, the series of biting cycles can be combined to give a single mean biting cycle by adding the twenty-four separate sets of figures corresponding to the twenty-four hours. The figures for all four platforms may be combined in the same way or kept separate as desired. The figures given in Table V represent the mean biting cycles of the species concerned and are based on all four platforms combined. To facilitate comparison, the hourly totals have been reduced to percentages of the grand total. Fig. 2 shows the curves based on these figures. In order to smooth and simplify them they have been based on two-hourly aggregates. This has necessitated choosing between curves based on the assumption that the mosquitos were collected at the end of the odd hours (17.15, 19.15, 21.15, etc.), or at the end of the even hours (16.15, 18.15, etc.). In each case the choice made has been such as to give the most faithful picture of the biting cycle as revealed by the hourly figures but the method of grouping inevitably leads to some loss of significant detail and the curves should be regarded only as diagrams.

Aëdes grahami.

This was easily the most abundant tree-top biter at Itowolo. Its mean biting curve (fig. 2) resembled to some extent that of *Anopheles gambiae* (Mattingly, 1949) but the peak biting time occurred an hour earlier. At this time marked peaks were recorded from both the upper platforms but the peak on platform II occurred an hour earlier still. The peak hour was not constant from catch to catch. Haddow (1945 & 1947) gives small figures from Uganda which appear to indicate a peak occurring during the 4-hour period 22.00–02.00 hours.

TABLE IV.

Seasonal distribution of mosquitos at Itowolo.

Catch No.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total
						1945											1946						
Date	14	June 21	28	5	July 12	19	26	Aug 2	9	23	Sep 6	20	Oct 4	24	Nov 21	Feb 13	Mar 13	Apr 3	17	May 1	15	Jun 12	
An. paludis	17	8	8	3	6	2	3	2	8	29	6	33	31	36	73	16	9	2	2	3	3	1	301
An. moucheti	0	12	17	12	12	8	17	13	12	17	7	3	3	1	6	0	0	2	0	4	3	2	151
Aëdom. africana	2	1	3	12	2	2	0	5	6	0	10	1	5	2	1	0	1	5	0	2	0	0	60
Aë. grahami	12	38	80	18	29	46	53	11	5	80	4	314	185	116	8	0	0	1	1	0	1	81	1,083
Aë. nigricephalus	6	13	23	34	23	25	6	15	32	38	23	17	64	3	0	0	16	19	19	27	13	8	424
Aë. irritans	21	34	19	14	15	6	5	6	4	7	0	3	6	0	0	0	2	4	9	1	2	3	161
Aë. circumluteolus	0	2	3	0	0	0	0	0	1	5	3	54	22	1	9	2	0	0	1	0	0	0	103
Aë. flavicollis	65	99	95	82	45	86	49	27	20	2	3	5	6	21	9	0	5	18	7	3	9	39	695
Aë. taylori	27	47	71	33	29	38	37	11	22	1	0	5	4	23	3	1	5	0	3	0	7	17	384
Cu. thalassius	1	1	1	0	0	0	0	0	0	0	0	0	69	17	0	50	6	11	5	0	0	0	161

TABLE V.

Hourly and 2-hourly biting cycles at Itowolo.

Time (L.M.T.)	A. paludis. %	%	A. moucheti %	%	Aëdom. africana %	%	Aë. grahami %	%	Aë. nigricephalus %	%	Aë. irritans %	%	Aë. circumluteolus %	%	Aë. flavicollis %	%	Aë. taylori %	%	Cu. thalassius %	%
16.15	0·3	1·3	0·0	0·0	0·0	0·0	0·0	0·2	2·4	7·3	3·7	·6	3·9	13·6	0·1	0·1	0·3	0·3	1·2	1·2
17.15	1·0		0·0		0·0		0·3		4·9		9·9		9·7		0·0		0·0		0·0	
18.15	1·0	7·6	0·0	0·0	0·0	8·3	0·4	0·7	6·8	14·3	14·9	24·8	15·5	19·4	0·1	0·1	0·0	0·0	1·2	1·2
19.15	6·6		0·0		8·3		1·2		7·5		8·0		3·9		14·1		11·5		18·0	
20.15	2·7	10·7	2·0	5·3	38·3	53·3	3·0	4·2	9·0	14·9	1·2	9·2	1·0	7·8	13·2	27·3	11·5	23·0	8·1	26·1
21.15	8·0		3·3		15·0		3·0		5·9		1·9		6·8		9·4		9·4		10·6	
22.15	7·6	16·9	4·0	7·3	8·3	11·6	6·9	9·9	5·2	13·2	1·9	3·8	8·7	12·6	7·5	16·9	9·6	19·0	8·1	18·7
23.15	9·3		3·3		3·3		10·0		8·0		0·6		3·9		8·9		10·2		6·8	
00.15	12·3	21·9	2·6	7·2	13·3	16·6	11·5	21·5	5·9	12·5	3·1	3·7	7·8	9·7	6·3	15·2	6·0	16·2	12·4	19·2
01.15	9·6		4·6		3·3		10·4		6·6		1·2		1·9		7·3		8·9		5·0	
02.15	8·6	15·2	8·6	20·5	3·3	5·0	12·7	23·1	3·5	9·6	0·6	1·8	1·9	1·9	7·3	14·6	7·8	16·7	5·0	10·0
03.15	6·6		11·9		1·7		12·9		6·1		1·2		0·0		9·4		7·6		4·3	
04.15	6·6	11·9	12·6	41·7	3·3	5·0	17·2	30·1	5·0	10·4	3·1	4·3	3·9	5·8	10·6	20·0	9·4	17·0	7·5	11·8
05.15	5·3		29·1		1·7		8·0		5·4		1·9		3·9		4·5		6·8		4·3	
06.15	8·6	9·3	10·6	11·3	0·0	0·0	0·6	8·6	2·8	6·8	6·2	8·1	2·9	5·8	1·2	5·7	1·3	8·1	1·2	5·5
07.15	0·7		0·7		0·0		0·4		4·0		14·9		2·9		0·0		0·0		2·5	
08.15	1·7	2·7	1·3	2·0	0·0	0·0	0·2	0·6	3·3	5·9	9·3	24·2	6·8	10·7	0·0	0·0	0·0	0·0	0·6	3·1
09.15	1·0		0·7		0·0		0·3		2·6		5·6		3·9		0·0		0·0		0·6	
10.15	0·7	0·7	0·0	0·0	0·0	0·0	0·3	0·6	0·7	1·6	2·5	8·1	2·9	5·8	0·0	0·0	0·0	0·0	0·6	1·2
11.15	0·7		0·0		0·0		0·0		0·9		3·1		2·9		0·0		0·0		0·0	
12.15	0·7	0·7	0·7	1·4	0·0	0·0	0·3	0·3	0·2	1·1	0·0	3·1	0·0	2·9	0·0	0·0	0·0	0·0	0·0	0·0
13.15	0·0		0·7		0·0		0·2		0·9		0·6		1·9		0·0		0·0		0·6	
14.15	0·3	1·0	2·0	3·3	0·0	0·0	0·1	0·3	1·9	2·4	2·5	3·1	1·9	3·8	0·0	0·0	0·0	0·0	1·2	1·8
15.15	0·7		1·3		0·0		0·2		0·5		1·9				0·0		0·0		0·0	
Total	99·9	99·9	100·0	100·0	99·8	99·8	100·1	100·1	100·0	100·0	99·8	99·8	99·8	99·8	99·9	99·9	100·3	100·3	99·8	99·8

Fig. 2.—Biting cycles at Itowolo. The curves on the left are based on the odd hours, those on the right on the even hours.

A ëdes flavicollis.

The mean biting curve is similar in general type to that of *A ëdes africanus*, another tree-hole breeder (Mattingly, 1949). It shows, however, less concentration of biting into the peak hour and a relatively higher biting activity during the hour following and during the remainder of the night. There is a small morning peak but depression of activity begins early. The comparison with *A. africanus*, at both ends of the cycle suggests a greater sensitivity to light. Only one mosquito of this species was taken between 05.15 and 17.15.

A ëdes taylori.

The mean biting cycle very closely resembled that of *A. flavicollis* to which this species is nearly related.

A ëdes nigricephalus.

This species, like all the others so far considered, showed its maximum biting activity between sunset and sunrise but it had a much higher level of daytime activity and almost 30 per cent. of the total catch was taken during the hours of daylight (06.15–18.15). It was thus surpassed as a daytime biter only by *A. irritans* and *A. circumluteolus.* Kerr (1933), working on the ground and during the hours of darkness only, recorded peak activity during the hour ending 19.15 but his figures are very small.

Anopheles paludis.

This resembled *Taeniorhynchus africanus* (Mattingly, 1949) in having a rather indeterminate biting peak during the middle part of the night. Haddow (1945) gives 4-hourly figures indicating a rather earlier peak in Uganda and Kerr (1933), whose figures include a small proportion of *A. coustani* var. *ziemanni*, recorded peak activity during the hour ending 19.15.

A ëdes irritans.

This species, with its remarkably symmetrical bimodal biting curve, showed maximum activity just before sunset and about an hour after sunrise. With the possible exception of *A. circumluteolus*, it was the most active daytime biter taken at Itowolo. Kerr (1933) gives figures for night-time catches made on the ground only. He did not start his catches until 19.00 hours, Nigerian Standard Time (18.15 hours L.M.T.), i.e. an hour after the peak biting time at Itowolo, and he obtained his peak during the first hour of his catches. He finished his catches before the time of the morning peak at Itowolo of which his figures give no indication.

Anopheles moucheti var. *nigeriensis.*

The mean biting curve belongs to the same general type as that of *Anopheles gambiae* and, on the ground only, *A. hargreavesi* (Mattingly, 1949) and this type may prove to be characteristic of the subgenus *Myzomyia*. In *A. moucheti*, however, activity is much lower over most of the night and, in consequence, there is a very pronounced biting peak during the hour or so before sunrise. The figures given by Kerr (1933) for *A. hargreavesi* included a small number of *moucheti* but it is not possible to separate them out. At Itowolo the peak was well marked on the three lower platforms. The numbers taken from the top platform were too small to be significant.

A ëdomyia africana.

This species appears to be very sensitive to light since the well-marked evening peak did not occur until an hour later than that shown by other species with a similar type of curve. No specimens were taken before sunrise or after sunset. The mean biting curve most closely resembled those of *A. (Diceromyia) flavicollis* and *A. taylori*,

differing at the evening end mainly in the low level of activity during the hour ending 19.15 and the corresponding high concentration of activity in the following hour. The distribution of the two *Diceromyia* species over the 2-hour period 18.15–20.15, however, suggests that most activity probably took place during the latter part of the first hour, so that the difference may not have been so great as it appears.

Aëdes circumluteolus.

Haddow and others (1947) give moderately large four-hourly figures for this species from Uganda. In general, the agreement with those obtained at Itowolo is good, the principal difference being a rather higher level of daytime activity in Uganda. Nevertheless this species was second only to *A. irritans* as a day biter at Itowolo and was even slightly more active than that species during the most severe part of the day. Kerr (1933) gives figures for *A. lineatopennis* which probably refer to this species but they are based on night-time catches only and are very small and of no value for comparison.

Culex thalassius.

Figures given by Kerr (1933) indicate a morning peak for this species. They were taken from the ground only. At Itowolo the figures from the ground platform only also indicate a morning peak but on the upper platforms, where most of the biting occurred, there was a well-marked evening peak and this characterises the curve as a whole. The figures are too small for detailed analysis.

Vertical Distribution.

Table VI shows the percentage of the total catch of each species at each platform.

Anopheles paludis.

The detailed figures for this species most closely resemble those for *Taeniorhynchus africanus* (Mattingly, 1949), from which it differed mainly in being rather more abundant on the ground.

TABLE VI.

Vertical distribution at Itowolo.

Platform	An. paludis	An. moucheti	Aëdom. africana	Aë. grahami	Aë. nigricephalus	Aë. irritans	Aë. circumluteolus	Aë. flavicollis	Aë. taylori	C. thalassius
	%	%	%	%	%	%	%	%	%	%
IV	4·3	2·0	56·7	46·8	5·7	1·2	0·0	48·6	36·5	25·5
III	7·0	14·6	30·0	35·4	1·9	1·9	1·0	37·3	40·6	27·3
II	24·3	49·0	13·3	16·4	5·9	8·1	8·7	13·2	20·1	14·9
I	64·5	34·4	0·0	1·4	86·6	88·8	90·3	0·8	2·9	32·3
Total	100·1	100·0	100·0	100·0	1C0·1	100·0	100·0	99·9	100·1	100·0

Anopheles moucheti.

The small figures available for this species, and their subdivision in order to obtain vertical distribution data, render the occurrence of maximum numbers on platform II of doubtful significance.

Aëdomyia africana.

Although only small numbers of this species were taken, the greatest numbers occurred regularly on the two upper platforms. It appears to be a typical tree-top biter. It is interesting to note that its vertical distribution resembles that of *Aëdes flavicollis* ; its biting cycle also appears to resemble that of *flavicollis* more than others with the possible exception of *Aëdes taylori.*

Aëdes grahami.

This also appears to be a typical tree-top biter. It was more abundant on the top platform than on the third in every catch except the 4th, in which there were 39 from platform III and 29 from platform IV, and the 22nd which yielded only one *grahami* and that from platform III.

Aëdes nigricephalus, irritans and *circumluteolus.*

The very large proportions of these species occurring on the ground reflect their high degree of daytime activity. All species taken at Itowolo, whatever their vertical distribution during the night, were very largely confined to the ground during the daytime. The small numbers of *A. nigricephalus* taken on the upper platforms render the apparent inversion between platforms III and IV of very doubtful significance. Haddow & others (1947) give good figures for *A. circumluteolus* from two localities in Uganda. They obtained 96 per cent. on the ground at Mongiro and 89 per cent. on the ground at Mamirimiri.

Aëdes flavicollis and *A. taylori.*

The well-marked difference in vertical distribution between these species is interesting in view of their close relationship and the similarity of their biting cycle. *A. flavicollis* was more abundant on the top platform than on the third in all the larger catches except the 2nd in which 32 were taken from platform III as compared with 23 from platform IV. In the larger catches of *A. taylori* the numbers taken on platforms III and IV respectively were 11 and 11 in the 2nd, 15 and 26 in the 3rd, 21 and 30 in the 4th, 14 and 13 in the 5th, 12 and 7 in the 6th, 19 and 12 in the 7th and 26 and 9 in the 8th.

Culex thalassius.

The occurrence of maxima on the ground and the 3rd platform may indicate two different types of behaviour within this species and this is also suggested by the biting cycle (see above). The figures, when subdivided, are, however, very small and the species occurred in significant numbers in only two catches. It is therefore by no means improbable that neither the biting cycle nor the vertical distribution is of a very sharply defined type and that larger figures would be required to give a satisfactory picture of either.

General Discussion.

The expression "biting cycle", as a term in general use, has been employed throughout the present paper. That the phenomena so named represent merely a variation in the urge to bite is, however, very doubtful. The cycle is in fact a record of the frequency with which the mosquitos come into contact with the bait and, as such, may be accounted for either by the urge to bite or by changes in the general level of activity of the mosquito and, in particular, of its speed and duration of flight. The distinction is important since biting *per se* may well be affected by the difference between the skin temperature and humidity of the bait (Parker, 1948) and the temperature and humidity of the surrounding atmosphere. Light, on the other hand, seems unlikely to affect biting except in so far as it affects the general level of activity of the mosquito. Finally, there is the possibility that intrinsic rhythms, affecting the mosquito independently of changes in its physical environment, may be to a greater

or lesser extent involved. The possibility that all three factors are involved and that their degree of interdependence may vary with the species and with varying conditions cannot be excluded. As an initial simplification it is not, however, unreasonable to picture the biting cycle as representing the frequency with which a population of mosquitos in random flight comes within the range of attraction of the bait. If this is so then the form which it takes will be governed by the effective velocity of the mosquitos, a rate compounded of their actual velocity of flight and the relative lengths of the periods during which they are at rest and on the wing, and the range of attraction of the bait which may vary independently. If the general level of activity of the mosquito possesses the importance which such a picture would imply, then it might be expected that the biting cycle would be related to changes in vertical distribution, since both would be affected by the same factors. Attempts have been made to relate these two aspects of mosquito behaviour as revealed by the data obtained at Itowolo and some interesting results have been obtained, but it is clear that it would be unwise to put forward even a tentative hypothesis in the absence of more detailed studies.

There is evidence that changes in the vertical distribution of certain species are associated with swarming and if this is so then it would introduce a further complication. Unfortunately, the phenomenon of swarming has been very little studied in African mosquitos but extensive researches on the subject have been carried out by Nielsen and Greve in Denmark. A preliminary account of this work was presented to the International Entomological Congress at Stockholm by the first-named author from which it is clear that the cyclical activities involved bear a striking resemblance to those under discussion in the present paper. There is every reason to hope that light will be shed on the problems arising from the work at Itowolo by the full account of Nielsen and Greve's work which is at present in preparation.

With regard to the biting cycle, the inhibitory effect of light, even of quite low intensities, seems to be clearly indicated by the sudden cessation of activity at daybreak when changes in temperature and humidity are negligible. It might be inferred that the sharp rise in activity which most species exhibit at nightfall is due to the removal of this inhibitory influence but such an inference cannot at present be supported by direct evidence since changes in temperature and humidity are quite pronounced during the critical period. That light of a certain preferred intensity (or even wave length) may have a stimulatory effect is not proven, but it appears to afford the simplest explanation of the behaviour of such species as *Anopheles moucheti*, which has a strongly marked peak associated with the period of morning twilight. Variations on this type of cycle, such as those shown by *A. gambiae* and *A. hargreavesi*, might be accounted for by different effects of temperature and humidity during the night when these are still undergoing fairly marked changes.

Finally, it should not be forgotten that environmental factors are not of necessity the only ones which need to be taken into account. Experience in the laboratory (Mattingly, 1946 ; Seaton & Lumsden, 1941) indicates that mosquitos feed most readily after a certain quite circumscribed period has elapsed since emergence from the pupa. If this is also true in nature then the biting cycle may be related to some extent to the breeding cycle and in particular to the time of emergence from the pupa. The first ten catches at Itowolo were made at intervals of exactly one week and it was observed that the total number of *Aëdes africanus* taken in each catch followed closely the proportion occurring on the top platform in the preceding week. The accompanying figure (fig. 3) shows the curves obtained from the last nine of these catches (the first was incomplete). It will be seen that over the first seven catches, which were those in which *A. africanus* was most abundant, the agreement was remarkably close. Catches later than the tenth were made at longer intervals. The agreement between the two curves appears too close to be coincidental. It

appears to be explained in part by the presence of a sibling species, *Aëdes* (*S.*) *pseudo-africanus* (Chwatt, 1949) distinguishable from *africanus* only on the male terminalia and possibly on small differences in the scutal markings. Major Chwatt kindly examined my collection at Yaba and found a single undissected male of this species and a search of the British Museum collection revealed several others from Yaba and Lagos placed under *africanus*. Preliminary observations suggest that it is more of a ground-biter than *africanus* and it is possible that it has a rather more diffuse type of biting cycle. This would explain discrepancies in the behaviour of " *africanus* " in Nigeria and Uganda noted in the first part of the present paper. Whether the phenomena under discussion can be wholly explained in this way can only be decided when the biting cycle and vertical distribution of the new species have been fully worked out. It seems not unlikely that some relationship between vertical distribution and the breeding cycle is also involved.
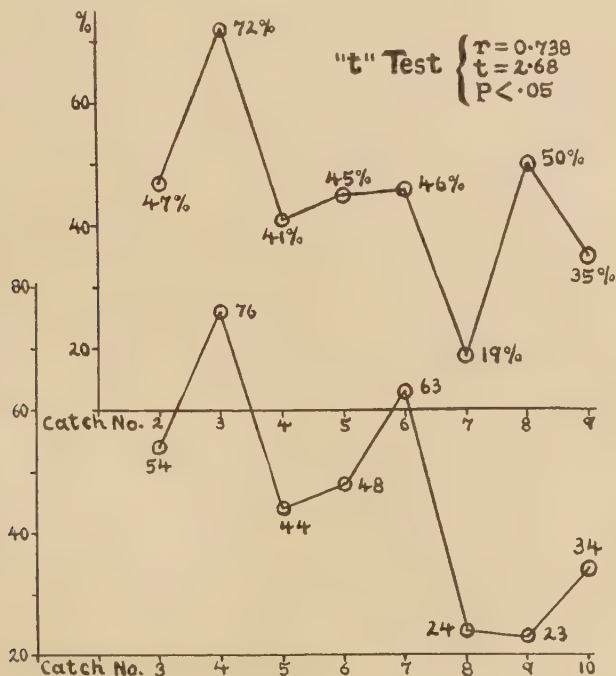
Fig. 3.—Apparent correlation between total catches of *Aëdes africanus* and vertical distribution during the previous week. The lower graph shows the numbers taken in each catch, the upper the percentage of the previous catch occurring on Platform IV.

Summary.

The present paper is the second of two dealing with field studies carried out near Lagos, Nigeria. The first paper covered four of the most abundant species encountered and the second is concerned with the remaining forty-eight. These are listed and the data relating to the thirty-eight least abundant, with the exception of two which were taken as males only, are given in tabular form. The other ten are dealt with at greater length. Males of twenty-six species were taken and in the case of three of these species they were so abundant as to create the impression that a swarming activity was involved. In the case of the ten more abundant species seasonal distribution curves are given based on average monthly catches and an attempt is made to relate these to variations in rainfall. The biting curves which

are next discussed show a greater variety of types than those described in the first
paper. In general, they are characterised by a fairly sharp peak associated with
morning or evening twilight, but some have a more or less pronounced peak at both
ends of the cycle and so correspond to the " eo-crepuscular " type of Haddow (1945).
Anopheles paludis, like *Taeniorhynchus africanus* (described in the first paper) has
an ill-defined period of maximum activity during the middle of the night. The
necessity of sub-dividing the figures in order to obtain a picture of the vertical dis-
tribution of the various species led in one or two cases to anomalous results, but in
general a clear and fairly convincing picture was obtained. None of the species
dealt with in the present paper was sufficiently abundant to afford reliable data on
hourly variations in vertical distribution. In the general discussion, which embodies
such inferences as it has seemed permissible to draw from the data, attention is drawn
to the fact that the so-called " biting cycle " does not, in all likelihood, depend
exclusively on variations in the urge to bite, but is more probably an expression of
cyclical changes taking place in the general level of activity of the mosquito. The
possible relationship of such changes to changes of a similar kind in the physical
environment is discussed, and it is pointed out that intrinsic rhythms in the mosquito,
perhaps related to the breeding cycle, may also be involved. , Some data are presented
which, while partly explained by the presence of a sibling species, may also indicate
an interrelationship between the breeding cycle and vertical distribution.

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THE SPEED OF ACTION OF INSECTICIDAL SPRAYS AND DEPOSITS AND ITS USE IN ASSESSING THE BIOLOGICAL EFFICIENCY OF BHC, DDT AND PYRETHRUM.

By D. S. KETTLE, M.Sc. (Lond.).

The Cooper Technical Bureau, Berkhamsted, Herts.

CONTENTS.

Introduction.

In recent years commercial fly-sprays have been revolutionised by the development of powerful synthetic insecticides such as DDT, 2 : 2 bis (parachlorophenyl)-1 : 1 : 1 trichlorethane, and benzene hexachloride, 1 : 2 : 3 : 4 : 5 : 6-hexachlorocyclohexane. A fly-spray is conveniently rated on its performance in the Peet-Grady test (Anon., 1946) in which it has to satisfy two basic requirements. Firstly, when used at a reasonable dosage it must produce a high mortality among the treated flies at the end of 24 hours and it should act as quickly as possible, i.e. have a high knock down in 10 mins. Formerly the Peet-Grady rating depended on the mortality at the end of 24 hours and only indirectly on the speed of action—since flies which were normal at the end of 10 minutes were assumed to survive and were not collected

(Anon., 1945). Nowadays the addition of as little as 0·1 per cent. DDT to a fly-spray will ensure 100 per cent. mortality. Therefore to meet this change the Peet-Grady test has been modified so that the 10-minute knock down (K.D.) of a fly-spray must be within −2 per cent. of that of the official test insecticide (O.T.I.) before it is eligible for a Peet-Grady rating (Anon., 1946). It is apparent that, in the future, interest in fly-sprays and their constituents will be centred more on the speed of action than on the mortalities they produce.

When preparations containing either DDT or BHC are applied to a surface, they leave behind a deposit which remains insecticidally active for a long period of time, the exact duration of which depends upon the conditions of application. Quite brief periods of contact, e.g., one minute, with these films produce high mortalities of *Musca domestica*, L. (Lindquist & others, 1945) so that it is difficult to compare the relative efficiencies of residual deposits by the construction of mortality/dosage (time of exposure) curves owing to the technical problem of exposing flies to deposits for sufficiently short periods of time. However, deposits can be compared by studying their speeds of action, i.e. the rate of K.D. (Parkin & Green, 1947).

It was decided, therefore to investigate the speed of action of various fly-spray constituents as sprays and deposits on the common house-fly (*Musca domestica*). Almost at once, two factors emerged which influenced the results considerably, the intensity of illumination and the relative proportion of the sexes among the treated flies. The first variable was eliminated by a more stringent technique but, as the second was more or less uncontrollable, a correction was introduced to allow for any deviation from the " normal " 1 : 1 sex ratio.

As work proceeded it became clear that, as in the case of the mortality, the speed of action was influenced by the dosage and concentration of the insecticide, and therefore by keeping the dosage constant it was possible to calculate the concentration of an insecticide from its speed of action. This was found to be of great value for the rapid estimation of the biological efficiency of samples of DDT, BHC and pyrethrum, particularly since the chemical determination of the insecticidally active constituent or isomer, i.e. para para content of DDT, gammexane* content of benzene hexachloride and pyrethrin content of pyrethrum, is either tedious or impossible.

The methods used and some of the results obtained are set out below.

Technique.

Fly Breeding.

The flies were bred according to a method described in 1946 (Anon.), except that the composition of the larval medium was :—

Alfalfa	...	...	150 grms.	Malt	50 cc.
Bran	...	...	600 ,,	Water	1,600 ,,
Yeast	...	...	20 ,,		

Batches of 100 pupae were placed in cloth-covered wire cages 12 in. high and 6 in. diam. and kept for seven days until the adults that emerged were 4–6 days old and ready for experimental use. For a few early experiments the pupae were put up in 500's in larger wooden cages, but it was found that the resistance of successive samples of flies from one cage differed considerably (see p. 413). Therefore the entire contents of a cage had to be used for each treatment and since the assessment of K.D. is best made on less than a hundred flies, it involved much work without the comparable data that replication usually brings.

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*Throughout this paper gammexane will be used to refer to the gamma isomer of benzene hexachloride.

The flies were fed daily on reconstituted dried full-cream milk (proprietary brand ' Lacta ')* and kept in the breeding room at 30°C. and variable relative humidity until the evening before treatment when they were given freshly prepared milk and transferred to the experimental room at 26°C. and variable humidity. Although there was no humidistatic control in either of these rooms their relative humidities remained remarkably constant throughout any particular day, *e.g.* max. 53, min. 47, mean 50 per cent., and only changed slowly over a period of several weeks as the outside temperature altered.

The flies were conditioned overnight (at least 17 hours) to the lower temperature (26°C.) and to an intensity of illumination of 20 foot-candles as measured by an " Avo "† lightmeter placed at the centre of the cage and the consistency of the results suggested that this exposure was adequate (see p. 410).

Experimental conditions.

i. *Spraying Conditions.*—All spraying experiments were conducted in a small chamber built to the specification of Kearns and March (1943) and the sprays were delivered under the following conditions :—

Spraying pressure	=	12·5 lbs./sq. in.
Dosage	=	0·8 cc. (0·4 cc. in each atomiser)
Rate of delivery—Atomiser 1	=	1·0 cc. in 4·6±0·05 sec.
,, 2	=	1·0 cc. in 4·8±0·05 sec.

Glass atomisers were used in the chamber since they were easily cleaned by immersion in chromic acid and minute particles of dust, which might influence the delivery rate, were quickly noticed. It was found that the delivery rate of an atomiser was more readily altered by adjusting the position of the fluid aperture with respect to that of the air jet than by heating or filing away the tip as suggested bh Kearns and March (1943). The suitability of the atomisers was further checked by spraying equal volumes of dye through each sprayer simultaneously and noting thy distribution of the droplets on the upper half of the chamber and the two end doorse When such a test was carried out with atomisers 1 and 2 the droplets at each end. of the upper half of the chamber were small and increased in size towards the centre where there was a band of large droplets—it is important that this band should not be eccentric—and on each end door the droplets were evenly distributed over the upper half.

The end walls and bottom half of the spray chamber were covered with clean paper before each test. This was thrown away after each spraying and the chamber cleaned with 10 per cent. acetone in 90 per cent. alcohol to remove any traces of residual insecticides.

During spraying the chamber was illuminated by three overhead electric light bulbs to give a mean intensity of illumination of 27–29 foot-candles. This figure was derived from two sets of readings, one upper and one lower. Each set consisted of three observations made at the extreme left, centre and extreme right of the chamber. A typical series of readings were :—

Upper   31 ; 40 ; 30

Lower   22 ; 23 ; 20   Mean=27·7 foot-candles

ii. *Preparation of Residual Deposits.*—The work on residual deposits has been confined to three preparations of DDT :—

1. A solution of pure p.p. DDT in petroleum distillate.

2. A solution of technical DDT (80 per cent. p.p.) in petroleum distillate.

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3. A finely divided suspension of technical DDT prepared by wet grinding in a ball mill (particle size $<25\mu$).

In the case of the solutions, deposits of DDT were applied to aluminium panels by means of the spraying tower designed by Webb (1947). The amount of deposit was varied by altering the concentration of the insecticide sprayed, whilst the other conditions were kept constant at :—

Spraying pressure	$=25$ lbs./sq. in.
Volume of liquid	$= 5$ cc.
Time to spray 5 cc.	$=12 \cdot 5$–$13 \cdot 0$ secs.
Temperature	$=20°$C.
Period of exposure of panel to settling mist	$= 5$ mins.
Period between spraying	$=10$ mins.
No. of priming doses	$= 2$

From Table I given in Webb (1947, p. 215) it is clear that, when a 1 per cent. solution of a dye in petroleum distillate is sprayed under these conditions, the deposit is 25 mg./sq. ft. and it is assumed here that the deposit is directly proportional to the concentration of the solute. The treated panels were kept at room temperature whilst crystal formation proceeded. Panels sprayed with solutions of pure p.p. DDT appeared to " dry " more quickly than those sprayed with technical DDT, on which cloudy areas of small droplets were discernible. These plates were stored for a few weeks before use until they also appeared " dry."

Ball milled DDT is not suitable for application through the tower and so an alternative method was developed. The required amount of suspension was placed on a panel, made grease free by two washings in pure trichlorethylene, and spread as evenly as possible by means of a small camel hair paint brush. The concentration of the suspension was always adjusted so that the required amount of DDT was contained in 16 drops delivered from a standard pipette. These were evenly distributed over the panel and spread with a brush previously dipped into the suspension. In order to test the accuracy of this technique four sets of panels were prepared with a deposit of 25 mg./sq. ft. technical DDT ($=20$ mg. p.p. DDT/sq. ft.). Four tests were carried out on each set of panels (all the sets were tested on the same occasion) and the mean median K.D. times were $21 \cdot 5$; $21 \cdot 0$; $21 \cdot 3$ and $19 \cdot 9$ mins. from which it was concluded that this method of application was satisfactory but, as will be shown later (p. 424), this may not be necessarily true. For use the treated panels were fitted into the testing chamber described by Webb (1947). .

Factors affecting the Action of Sprays and Residual Deposits.

Effect of intensity of illumination.

i. *On Sprays.*—The first observation, which suggested that the intensity of illumination might influence the action of sprays, was made in an experiment on the effect of age on the resistance of *M. domestica*. Flies, 3–5 and 4–6 day-old, taken from separate cages both morning and afternoon, were sprayed with the Official Test Insecticide ($= 0 \cdot 1$ per cent. w/v pyrethrins) under natural illumination in the Kearns and March chamber. Three samples, totalling 300–400 flies, were taken from each cage. The results are set out in Table I where the mortality of the males has been omitted since it was very nearly 100 per cent. The difference between the percentage mortalities of 3–5 and 4–6 day-old flies was $3 \cdot 2$ per cent. in the morning and $3 \cdot 3$ per cent. in the afternoon yet between the morning and afternoon the percentage mortality of both groups shifted — 11 per cent. Temperature and humidity records taken during the period of experiment showed that these factors were not responsible and the only reasonable explanation was that the change in intensity of illumination had been the cause. In the morning it was snowing heavily and the experimental room was brilliantly illuminated by reflected light whereas in the afternoon the wintry sun was setting and the room was quite dull and it was noticed

TABLE I.

The change in percentage mortality of female *M. domestica* caused by an alteration in the natural illumination.

Age of Flies in Days	Morning		Afternoon		Difference between Morning and Afternoon
	Experimental Room Bright		Experimental Room Dull		
	No. of females	% Mortality	No. of females	% Mortality	% Mortality
3–5	173	54·3%	141	43·3%	—11·0%
4–6	167	57·5%	178	46·6%	—10·9%
Difference in % Mortality between 3–5 and 4–6 day-old Flies	+3·2%		+3·3%		

The flies had been sprayed with 0·1% w/v pyrethrins in the Kearns and March small chamber.

that the flies were sluggish. To test this an experiment was carried out with 4–6 day-old flies. They were conditioned to an intensity of illumination of 7 foot-candles for 1 hour before spraying commenced and then exposed to different intensities of illumination in the chamber during spraying. It was clear from the results that the intensity of illumination in the spray chamber markedly influenced the mortality, and that this effect was greatest at low intensities of illumination (Table II).

TABLE II.

Effect of intensity of illumination in spray chamber on percentage mortality of female *M. domestica*.

Mean Intensity of Illumination in Spray Chamber	No. of Females	% Mortality
12 foot-candles	192	33·3%
17 ,, ,,	184	54·3%
24 ,, ,,	155	58·7%
37 ,, ,,	173	60·5%

ii. *On Deposits.*—The speed of knock down of five sets of panels was assessed twice on one day. Of these, four sets were coated with 25 mg. ball milled technical DDT/sq. ft. and one had received a deposit of 25 mg. technical DDT/sq. ft. from petroleum distillate. The sets were exposed in pairs. The experimental room was kept in complete darkness except during the actual experiments of which six were carried out in the morning and four in the afternoon with a two hour interval, when the room was in darkness. During experiments the room was illuminated by a single 100-watt bulb and the cages were arranged so that they were equidistant from the source of light and were illuminated rather weakly from above. Since each experiment took an hour to perform the flies used in the first experiment received no illumination, those in the second received one hour illumination and those in the third received two. The differences between the median knock down times (K.D.$_{50c}$) of the duplicate experiments of each set of panels have been plotted against the differences in illumination, received by the flies before exposure (fig. 1). It is clear that the speed of K.D. is directly related to the illumination received by the flies

before testing.   That is, the longer the illumination the quicker the speed of K.D. within the limits of this experiment.

Before the implication of the previous experiment was fully appreciated, another was conducted under the same conditions, except that the flies were illuminated for one hour before the experiment began and the light was left on during the interval. In this experiment ten sets of panels were tested.   Five sets had deposits of $2\frac{1}{2}$, 5, 10, 20 and 40 mg. pure DDT/sq. ft. and the other five had received an equivalent amount of p.p. DDT deposited from a solution of technical DDT.   The sets of panels having equivalent p.p. DDT deposits were tested together.   When the $K.D._{.50c}$ values were plotted against the dosage, the resulting curve was chaotic (fig. 2A) but when these values were plotted in the order of testing a smoother curve resulted (fig. 2B).   It was clear that some factor was causing a greater change in the speed of K.D. than the variation in dosage.   It is suggested that this factor was the period of illumination before testing.
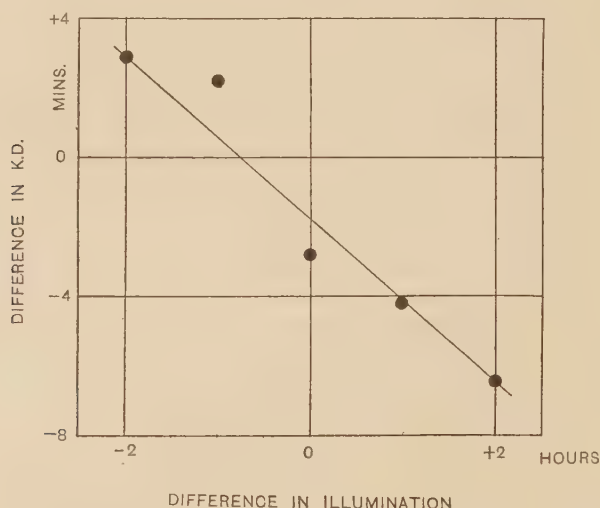
Fig. 1.—Effect on the speed of knock down of varying periods of exposure to a low intensity of illumination.   For details see text.

As a result of these observations the routine described on page 405 was adopted. It seems probable that the intensity of illumination affects the action of sprays and deposits by increasing the activity of the flies.   The researches of David (1946 a, b) and David and Bracey (1946) have shown that the amount of spray collected by flying insects is in direct proportion to their activity.   It is less clear why the intensity of illumination should affect the speed of action of deposits since it seems unlikely that an increase in activity would lead to an increase in dosage by bringing more of the body cuticle into contact with the insecticide.   It has been suggested that DDT, by blocking an enzyme system, brings about the accumulation of toxic products which cause the symptoms seen in treated insects (Hurst, 1945).   If this is true then it is highly probable that any factor increasing the activity of treated insects will lead to a more rapid accumulation of these products and therefore to a more rapid manifestation of toxicity, i.e., more rapid K.D.

Effect of Sex Ratio.

It has long been recognised that male and female *M. domestica* differ enormously in their resistance to insecticides (Miller & Simanton, 1938) so that the sex ratio

of a treated population is a most important variable which is difficult to control.   In calculations of the percentage mortality the influence of this factor can be eliminated, either by evaluating the percentage mortality separately for each sex and using the mean of these as the corrected percentage mortality of the treated population, or by using large numbers of flies, e.g. 500, in each test (Simanton & Miller, 1938). Neither of these methods is applicable to the assessment of K.D. by sprays or deposits because it is not possible either to sex the flies which are down at any particular instant or to assess accurately the percentage K.D. on large numbers of flies.   Therefore, experiments were conducted to determine the relative resistances of the two sexes to sprays and deposits.
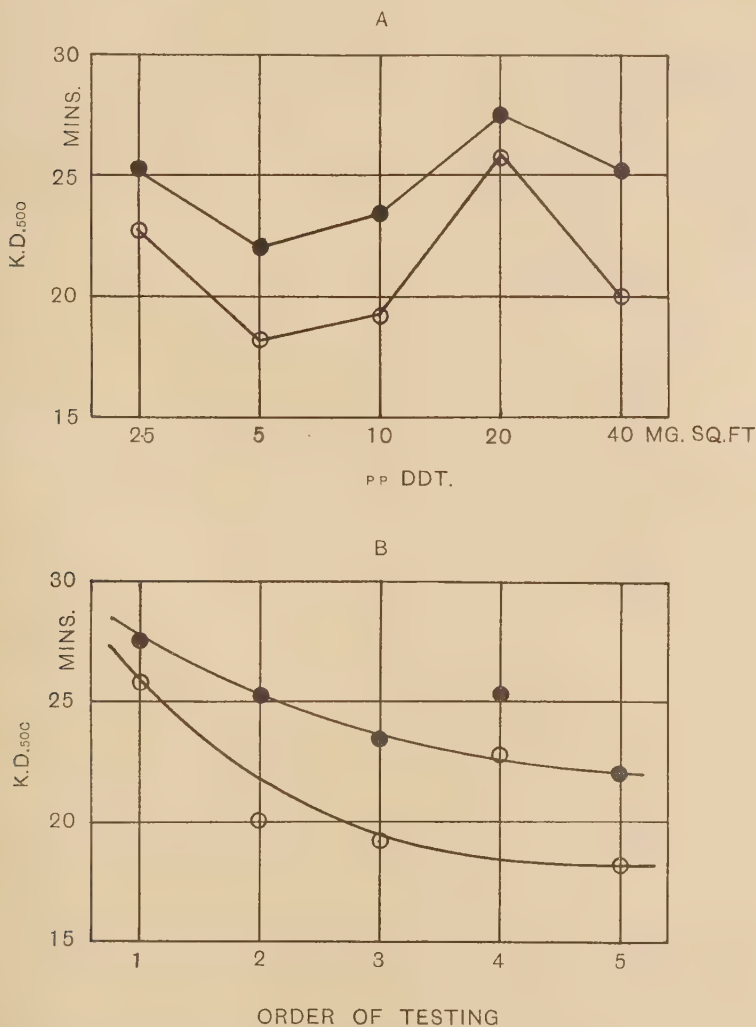
Fig. 2.—(A) Effect of concentration of p.p. DDT on knock down.
         (B) Effect of order of testing on knock down.

     For details see text.   Closed and open circles represent deposit put down from technical (80 per cent. p.p.) and pure (100 per cent. p.p.) DDT respectively.   K.D.$_{50c}$= corrected median knock down time.

i. *On Sprays.*—The day before the experiments were carried out the sexes of the flies were separated. This was achieved by cooling the flies for 10–15 minutes in a cold room (2°–3°C.), then turning them out on to a cold petri dish and rapidly sexing them. The flies were active and apparently normal again in 5–10 minutes, and were transferred to the experimental room at 26°C. where they were conditioned as usual. On the following day the flies were sprayed with 0·8 cc. of 0·05 per cent. w/v. gammexane in the spray chamber and to eliminate the effect of any factor other than sex influencing the results, the order of spraying was M1, F1, F2, M2, M3, F3, F4, M4, M5, F5, F6, M6, where M and F stand for male and female respectively. The number of K.D. flies was counted every minute and the results are set out in Table III.

It was found that there was a linear relationship between the percentage K.D. expressed in terms of probits (Bliss 1935, 1937) and the time after spraying (fig. 3). For each test the time taken to knock down half the number of flies (K.D.$_{.50}$) was read from the percentage K.D. (probit)/time curve and noted in Table III. The close agreement between the median K.D. times of replicate tests showed that the technique was quite well standardised when the effect of sex was absent. The mean K.D.$_{50}$ for male and female *M. domestica* was $6·25 \pm 0·15$ minutes and $8·95 \pm 0·13$ minutes respectively, and it is clear, therefore, that the K.D.$_{50}$ will be greatly affected by variations in the sex ratio.
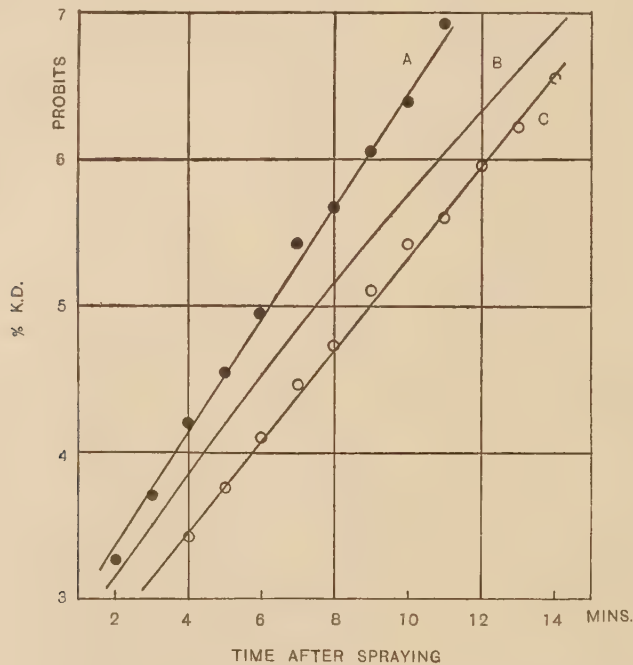
Fig. 3.—Speed of knock down of male (closed circles curve A) and female (open circles curve C) *M. domestica* by 0·05 per cent. gammexane. Curve B represents speed of knock down of a population with a 1 : 1 sex ratio. Curves A and C represent formulae 1 and 2 respectively. Data from Table III.

The following straight lines were fitted by eye to the observations plotted in fig. 3 :—

Male *M. domestica*   ...   ... $y=0·385t+2·595$ 1
Female *M. domestica* ...   ... $y=0·313t+2·197$ 2

where $y=$ per cent. K.D. (probit) and $t=$ time after spraying in minutes.

TABLE III.

Speed of K.D. of male and female *M. domestica* by 0·05 per cent. w/v gammexane.

(a) Time after Spraying in mins.	(b)	(c)	(d)	(e)	(f)	(g)	(h) Weighted Mean % K.D.	(i) Expected % K.D. from Formula I (male) and Formula II (female)	(j) Difference (h−i)
	Per cent. K.D. in Test No.								
	1	2	3	4	5	6			
2	4	5	4	2	4	5	4·1	5·1	− 1·0
3	14	7	8	8	3	15	9·7	10·5	− 0·8
4	22	17	35	16	18	20	21·2	19·4	+ 1·8
5	32	30	39	30	31	33	32·3	31·6	+ 0·7
6	50	42	54	46	50	46	48·0	46·3	+ 1·7
7	69	66	74	63	60	64	66·1	61·5	+ 4·6
8	78	74	83	71	72	71	74·9	75·2	− 0·3
9	88	84	93	84	81	81	85·2	85·7	− 0·5
10	93	92	93	89	92	91	91·8	92·7	− 0·9
11	98	98	97	95	97	98	97·3	96·7	+ 0·6
No. of Males ...	99	88	72	63	72	92	486		
K.D.$_{50}$ in mins. ...	6·0	6·4	5·6	6·4	6·4	6·3	6·25		
4	9	5	5	6	3	5	5·6	6·1	− 0·5
5	13	10	10	10	10	11	10·5	10·9	− 0·4
6	22	20	15	19	14	20	18·3	17·9	+ 0·4
7	38	21	26	32	26	34	29·5	27·3	+ 2·2
8	38	32	41	36	46	43	39·3	38·6	+ 0·7
9	57	51	56	45	64	49	53·8	50·9	+ 2·9
10	72	65	67	62	70	58	66·0	63·2	+ 2·8
11	78	67	75	76	73	66	72·5	74·2	− 1·7
12	83	82	82	85	89	77	83·2	83·3	− 0·1
13	89	88	93	92	92	78	88·8	90·0	− 1·2
14	99	90	98	97	95	85	94·0	94·4	− 0·4
No. of Females ...	79	82	61	78	91	74	465		
K.D.$_{50}$ in mins. ...	8·5	9·2	9·0	9·0	8·6	9·1	8·95		

These expressions are particular examples of the general formula $y=at+b$ in which a and b represent the slope and position of the line.   In a given set of conditions the constants a and b are determined by the insecticide and its concentration respectively.

From expressions 1 and 2 the speed of K.D. of a population consisting of 50 per cent. males and 50 per cent. females was calculated, and the K.D.$_{50}$ of such a population was 7·45 minutes.   By substituting this value for t into these expressions it was found that 7·45 minutes after spraying, 32 per cent. of the females and 68 per cent. of the males were K.D.   Therefore, if the K.D.$_{50}$ of a treated population with a normal 50 : 50 sex ratio is q minutes, than in the same time the percentage K.D. of a similarly treated population whose sex ratio is x per cent. males : y per cent. females, will be $0·68x+0·32y$. Hence, the influence of unequal sex ratios on the median K.D. can be eliminated by determining K.D.$_{50c}$ instead of K.D.$_{50}$, where $50c=0·68x+0·32y$; $x=$per cent. males and $y=$per cent. females in the treated population.

If it is true that the percentage K.D. (probit)/time relationship for each sex is a straight line, then the resistance of each sex, measured in terms of the standard deviation, is normally distributed, but since these lines are not identical the means are different.   Therefore, the resistance of a population of mixed sexes will have a
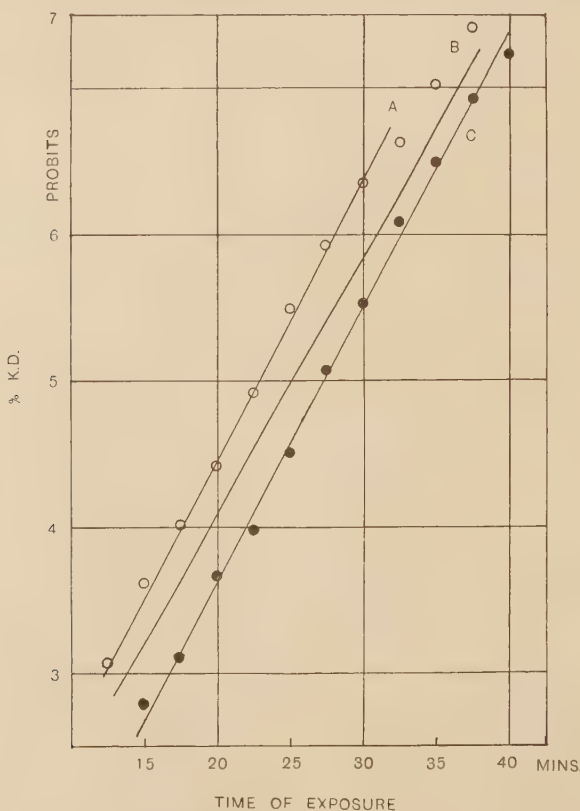
Fig. 4.—Speed of K.D. of male (open circles curve A) and female (closed circles curve C) *M. domestica* by a deposit of 20 mg.p.p.DDT/sq.ft.   Curve B represents speed of K.D. of a population with a 1 : 1 sex ratio.   Curves A and C represent formulae 3 and 4 respectively.

bimodal distribution and the percentage K.D. (probit)/time relationship will not be linear. This is confirmed by observations on mixed populations (*cf.* curve B fig. 3 and fig. 6).

However, since the curvature of this percentage K.D. (probit)/time curve between probits 4 and 6 is only slight, the determination of the K.D.$_{50}$ was made by plotting all K.D. observations between 10 and 90 per cent. and fitting a straight line to them by eye. Greater emphasis was attached to observations around the mean than at the extremes. From this line the K.D.$_{50c}$ was read. In a few instances the curvature was obvious from the observations and then a freehand curve was fitted.

ii. *On Deposits.*—The sexes of the flies were separated by the method described on p. 410, except that there was only one large cage for each sex and not six small ones. Two sets of panels were coated with a deposit of 20 mg. pure p.p. DDT/sq. ft. and each sex was exposed on each set alternately, in case there should be any difference between the sets. Since observations were taken every $2\frac{1}{2}$ minutes it was possible to conduct two experiments simultaneously, and the males and females were exposed at the same time. Four tests were carried out with each sex and the consolidated results are set out in fig. 4.

Here again, it is apparent that there is a linear relationship between percentage K.D. (probit) and the period after exposure. The following linear expressions were fitted by eye to the observations :—

Male *M. domestica*	...	...	$y=0\cdot191t+0\cdot625$	...	...	...	3
Female *M. domestica* ...	...	$y=0\cdot187t-0\cdot115$	...	...	...	4	

where $y=$ per cent. K.D. (probit) and $t=$time of exposure in minutes.

From these it was calculated that the K.D.$_{50}$ for males and females was 22·9 minutes and 27·4 minutes respectively and for a population with a 50 : 50 sex ratio it was 25·1 minutes. By substitution in expressions 3 and 4 it was found that 25·1 minutes after exposure, 66 per cent. of the males and 34 per cent. of the females were knocked down. Therefore, the value of 50c in this experiment was $0\cdot66x+0\cdot34y$, where x and y represent the percentages of males and females respectively in the treated population. However, this is very nearly the correction which was arrived at from experiments with gammexane sprays (see p. 412) so that in all the following experiments 50c was taken as equal to $0\cdot67x+0\cdot33y$.

One other interesting conclusion can be drawn from this experiment. When the percentage K.D. (probit)/time curves were constructed for each sample drawn from the cage, it was seen that the speed of action varied with the number of the sample (fig. 5). The median knock down times for the four samples of males were 21·3 ; 21·3 ; 23·5 and 28·8 minutes, whilst for the females the times were 25·1 ; 25·6 ; 28·7 and 30·3 minutes respectively. This change was not due to the mechanical removal of the deposit by the flies since, when repeated, the first samples of males and females were knocked down almost as rapidly as in this experiment. It was noticed that the flies in the third and fourth samples were rather sluggish—it is self-evident that when a cage is opened the more active flies will fly out first—and this suggested that the slow rate of knock down was due to the lack of activity of the flies. It has already been suggested that the intensity of illumination affects the rate of action of an insecticide by altering the degree of activity of the insect, and this appears to be another example of the same phenomenon, except that in this case the inactivity is due to the internal conditions of the flies, and not to the absence of external stimuli.

When only 100 flies were used in each Peet-Grady test it was usual to take successive samples from a cage of about 1,000 flies but, since it was realised that the resistances of these samples were not comparable, the tests of the unknown and standard were randomised. This difference between the samples was due in part to unequal sex ratio but it is clear from this work that sex was not the only factor involved.

The Use of the Speed of K.D. to assess the biological Efficiency of BHC, DDT and Pyrethrum.

Chemical analysis is used by insecticide manufacturers and others to check the quantity of constituents in preparations. The assumption behind the use of this method is that the biological efficiency of a given preparation is directly proportional to its chemical constitution. However, this is not always true since the presence of activators, such as Sesame oil (Eagleson, 1942 ; David & Bracey, 1944), or depressants, will raise or lower the biological potency of a preparation. Clearly, biological
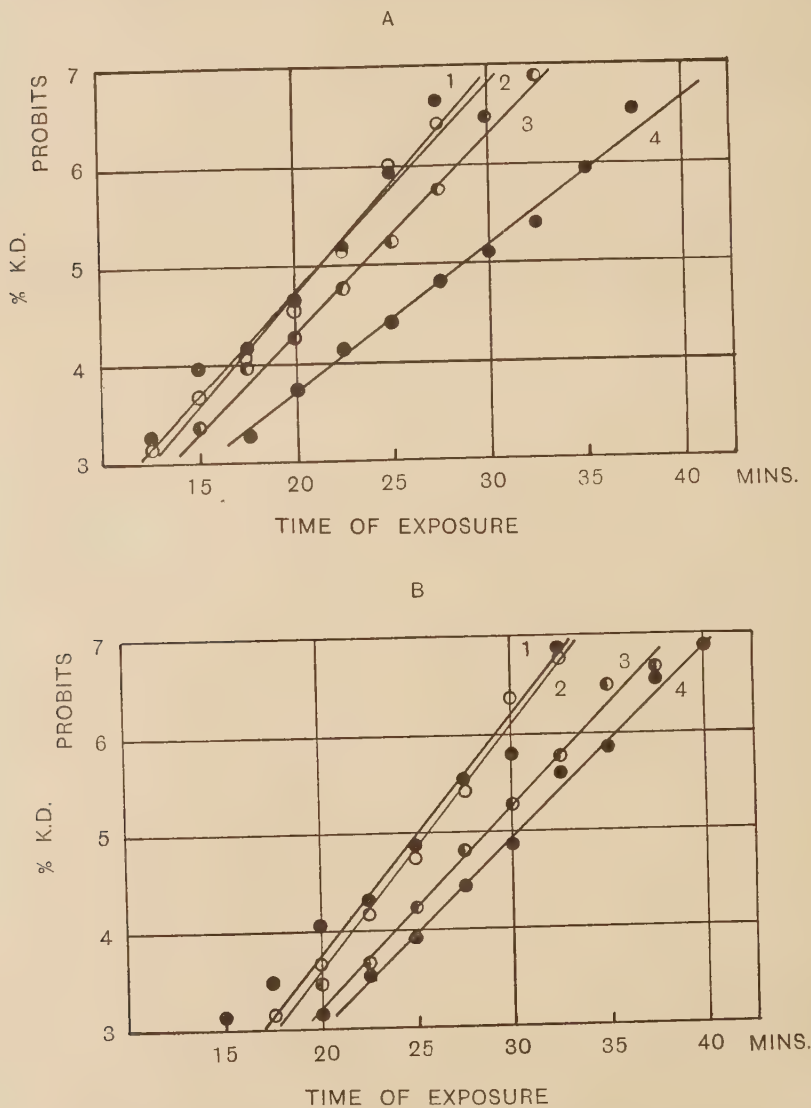
Fig. 5.—Speed of K.D. of successive samples taken from separate cages of male (graph A) and female (graph B) *M. domestica*. Closed circles represent 1st and 4th samples. Open and half-closed circles represent 2nd and 3rd samples respectively. Curves 1–4 indicate the numbers of the samples.

tests cannot determine the quantity of a particular chemical in a preparation, but they can evaluate the biological efficiency, and this, after all, is the more important value from the point of view of the consumer. For simplicity, throughout the following sections, this efficiency will be expressed in terms of the concentration of the known insecticidally active constituent.

Benzene Hexachloride.

Commercial BHC is a mixture of four isomers of which only one—the gamma—possesses outstanding insecticidal properties and this forms only about 10–12 per cent. of the crude product (Slade, 1945). So that, if, in the course of manufacture, some of the BHC is removed, as for example when the crude material is washed with an organic solvent, it becomes of paramount importance to estimate the gamma content of the processed material. Unfortunately, there is no chemical method for this, and so one has to rely on biological methods which usually involve the comparison of the percentage mortality produced by the unknown with those caused by suitable standards. When this comparison is effected by using BHC dust against a suitable insect, e.g. *Calandra granaria*, L., it is usual to wait six days before assessing the mortalities among the treated populations. This delay can often be ill afforded. If the comparison is made on a fly-spray a further difficulty arises, namely, that when the concentration of gammexane is sufficient to knock the flies down in a reasonable length of time, e.g. 10–15 minutes, the kill is always 100 per cent., and when the concentration does not give a complete kill it takes a long time to overcome the flies so that they can be collected. David (1946b) surmounted this difficulty by exposing the flies in a small cage which he suspended in the spray chamber. If a quick acting agent such as pyrethrins or Lethane 384 is added to the spray it will cause a certain mortality and be an additional source of error.

i. *Definition of Knock Down.*—It was, therefore, decided to explore the possibility of assessing the gamma content of a solution of BHC in petroleum distillate from its speed of action on *M. domestica*. When flies were sprayed with gammexane solutions the following sequence of events occurred. Firstly, they behaved normally for 2–3 minutes, then they fell to the bottom of the chamber but quickly walked or flew up again. As time passed, they fell more frequently, sometimes turning on to their backs, and remained longer on the floor of the chamber. Then they were seized in a fit of frenzied activity in which they spun around madly on their backs and it was impossible to count the number of flies on the floor.

When house-flies were liberated into the spray-chamber they congregated in the upper half and only one or two would descend to the floor. Since the chamber was essentially a horizontal cylinder, the floor was defined as the area enclosed by two parallel lines drawn 3 ins. either side of the middle line. All flies in that area were classified as "down," and all outside it as "up". When the state of hyperactivity supervened the number of flies "up" was counted.

ii. *Calculation of Gammexane Content of a Solution of BHC.*—The percentage K.D. (probit)/time data for 0·1, 0·05 and 0·025 per cent. gammexane are given in Table IV and fig. 6. It will be noticed firstly that, as suggested earlier (p. 413) in a population of mixed sexes this relationship is not linear ; and secondly, that there is close agreement between the observed speed of action of 0·05 per cent. gammexane and that expected from expressions 1 and 2. The corrected median K.D. times of 0·1, 0·05 and 0·025 per cent. gammexane are 6·0, 7·4 and 8·3 minutes respectively, and the simplest linear relationship between these values is :—

$$y = b - 3 \cdot 821 \, \mathrm{Log} \, x \quad \ldots \qquad \ldots \quad 5$$

where $y = $ K.D.$_{50s}$ in minutes.

$x = $ concentration of gamma isomer.

$b = $ a constant in this example $= 2 \cdot 279$.

TABLE IV.

Speed of K.D. of *M. domestica* by sprays containing various concentrations of pure gamma BHC.

Time after Spraying in minutes.	Percentage K.D. by		
	0·1% γ	0·05% γ	0·025% γ
3	12·1	9·9 (6·8)	9·6
4	23·7	13·7 (12·8)	15·7
5	35·7	21·8 (21·3)	22·1
6	50·8	31·3 (32·1)	30·2
7	66·3	44·0 (44·4)	39·3
8	77·8	58·1 (56·9)	50·0
9	84·5	67·9 (68·3)	59·2
10	91·2	76·5 (78·0)	68·0
11	93·9	82·9 (85·5)	75·2
12	96·3	87·5 (91·0)	81·6
No. of Flies...	1,264	1,247	1,207
% Males ...	51·6%	50·1%	56·0%
K.D.$_{50c}$	6·0 mins.	7·4 mins.	8·3 mins.
K.D$_{50c}$ from Formula 5 ...	6·1 mins.	7·25 mins.	8·4 mins.
Difference in mins.	— 0·1 mins.	+ 0·15 mins.	— 0·1 mins.

This table gives the consolidated data from 14 tests for each concentration made over a period of two months. The figures in brackets under 0·05% γ give the percentage K.D. expected from formulae 1 and 2.
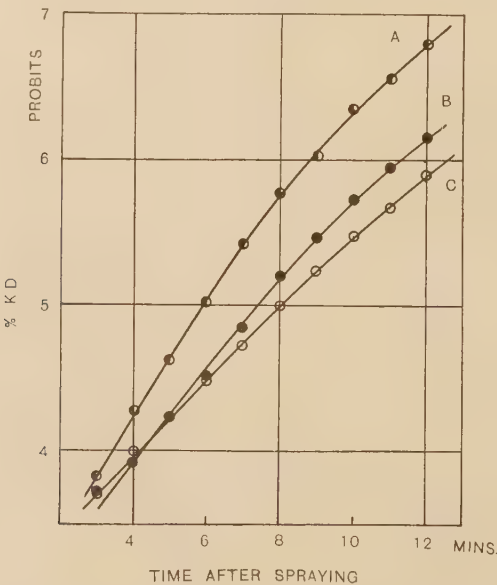
Fig. 6.—Speed of K.D. of *M. domestica* by 0·1 per cent. (half closed circles curve A); 0·05 per cent. (closed circles curve B) and 0·025 per cent. (open circles curve C) pure gammexane. Data from Table V.

It is well known by workers on *M. domestica* that there is considerable day to day variation in the resistance of flies to insecticides so that results are always related to a standard (Anon., 1946). In these experiments there was considerable variation in the median K.D. times of the three gammexane standards (Table V) so that it was not possible to assess the gamma content of a solution by substituting the value of its $K.D._{50c}$ into formula 5.

TABLE V.

Day-to-day variations in $K.D._{50}$ of 0·1, 0·05 and 0·025 per cent. pure gamma BHC.

Date	$K.D_{50c}$ in minutes of		
	0·1% γ	0·05% γ	0·025% γ
May 8	6·5	8·9	10·3
,, 8	7·1	7·5	7·8
,, 9	5·1	6·6	8·1
,, 9	6·7	8·3	10·2
,, 14	5·2	6·9	8·8
,, 14	6·5	8·3	9·7
,, 21	6·0	6·6	7·9
,, 22	6·0	8·2	9·0
,, 27	5·5	7·0	7·0
,, 29	7·2	8·1	8·9
June 19	4·4	6·1	7·3
,, 24	6·7	7·5	8·2
July 10	5·9	7·0	8·3

From formula 5 it can be seen that if the gamma content of a solution is halved, its $K.D._{50c}$ increases by $-3·821 \times \log.\frac{1}{2}$ ($=\overline{1}·6990$) which is equal to $+1·15$ minutes. It is possible that this increment may vary with the size of the median K.D. times, that is, the increment may be smaller when the flies' resistance is low, and the median K.D. times small, and larger when the median K.D. times are big. To decide if this were so the correlation coefficient between 31 pairs of variables was calculated. The independent variable was the mean of the median K.D. times of a solution determined for three strengths (S, $\frac{1}{2}$S and $\frac{1}{4}$S) and the independent factor was the mean difference $= \left(\dfrac{K.D._{50c}\frac{1}{4}S - K.D._{50c}S}{2}\right)$. The calculated correlation coefficient was $+0·17$ and P had a value of more than 0·1 so that there was no reason to suspect that the value of the increment depended upon the magnitude of the K.D. time, which suggests that although the value of b in formula 5 may vary from day to day the slope remains more or less constant.

This fact was utilised in the determination of the gammexane content of solutions. The $K.D._{50c}$ of each unknown solution was compared at three strengths (S, $\frac{1}{2}$S and $\frac{1}{4}$S) with the three standards (0·1, 0·05 and 0·025 per cent.) pure gammexane. If the mean difference between the median K.D. times of the unknown and standard be z minutes, then the logarithm of the concentration S is equal to the logarithm of the standard with which it is being compared (0·1 per cent. gammexane in this case) less $\dfrac{z}{1·15}(\log.\frac{1}{2})$ so that $\log.S = 1 - \dfrac{0·3010z}{1·15}$ For example, the following median K.D. times were obtained in an experiment :—

Concentration of unknown	K.D. $_{50c}$	Concentration of pure gammexane	K.D. $_{50c}$
S	4·3 mins.	0·1%	5·9 mins.
$\frac{1}{2}$S	6·2 mins.	0·05%	7·0 mins.
$\frac{1}{4}$S	7·4 mins.	0·025%	8·3 mins.

Mean difference K.D.$_{50c}$= $-$ 1·1 minutes so that log. concentration of

$$S=\log.\overline{1}-\left(\frac{0·3010\times-1·1}{1·15}\right)$$
$$=\log.\overline{1}+0·288$$

and $S=0·194$ per cent.

To test the accuracy of this method, six solutions of pure gammexane were prepared by a colleague and their gamma contents were estimated by this technique. The results are tabulated in Table VI. There was fair agreement between the estimated values and the actual gamma contents.

TABLE VI.

Comparison of actual and calculated concentrations of pure gamma BHC in petroleum distillate.

(a) Sample No.	(b) Calculated % γ	(c) Actual % γ	(d) Difference b − c	(e) % Difference d/c × 100
1	0·194	0·200	− 0·006	3%
2	0·098	0·100	− 0·002	2%
3	0·062	0·050	+ 0·012	24%
4	0·068	0·100	− 0·032	32%
5	0·134	0·150	− 0·016	11%
6	0·060	0·060	nil	0%
			Mean % Difference	=12%

TABLE VII.

Speed of K.D. of *M. domestica* by sprays containing varying concentrations of pure p.p. DDT.

Time after Spraying in minutes	% K.D. by p.p. DDT				
	3·2%	2·0%	1·6%	1·2%	0·8%
4	9·4	6·5	8·6	4·4	4·8
5	22·4	17·1	17·7	9·8	9·6
6	42·9	28·8	26·3	19·1	15·0
7	60·6	42·9	37·7	29·5	26·4
8	75·3	51·8	49·1	39·8	37·5
9	85·3	64·7	62·3	48·5	47·1
10	91·8	78·8	79·4	59·5	53·0
11	94·7	81·8	83·4	70·4	63·7
12		89·4	90·9	77·5	72·8
13		93·5	93·1	86·2	77·1
14			93·7	93·8	79·3
15					84·1
16					87·3
17					92·0
No. of flies ...	170	170	175	183	187
% Males ...	50·6%	45·9%	45·1%	53·0%	55·6%
K.D.$_{50c}$ in minutes	6·4	7·6	7·8	9·2	9·9
K.D.$_{50c}$ from Formula 6	6·4	7·6	8·1	8·8	9·9
Difference in minutes	0·0	0·0	−0·3	+0·4	0·0

Consolidated data from two tests.

This method allows one man to estimate the gamma content of three solutions in a day. It is felt, however, that the greatest source of error lies in the inadequacy of the sex correction factor when applied to small samples. This can be eliminated either by the duplication of tests or by using only one sex. The latter method would take less time than repetition and it is thought that the results would be more accurate than by duplication.

DDT.

Very few experiments have been carried out with DDT solutions by the author but these suggested that it should be possible to estimate the para para content of DDT solutions from their speeds of action. The results of two tests are given
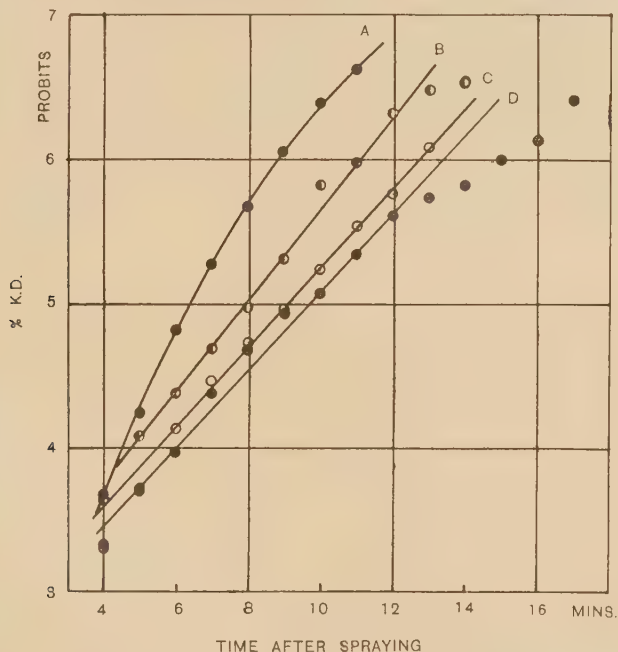
Fig. 7.—Speed of K.D. of *M. domestica* by 3·2% (closed circles curve A) ; 1·6% (half-closed circles curve B) ; 1·2% (open curve C) and 0·8% (closed circles curve D) p.p. DDT. Data from Table VII.

in Table VII and fig. 7. It will be seen that the speed of K.D. slows down as the concentration of p.p. DDT in the solution decreases. The simplest expression fitting these experimental results is :—

$$y = 9·3 - 5·8x \quad \dots \quad \dots \quad 6$$

where $y = $ K.D.$_{50c}$ in minutes.

$x = $ log. concentration of p.p. DDT in the solution.

There is close agreement between the experimental results and those calculated from expression 6 (Table VII). It will also be noticed that when the concentration of p.p. DDT in a solution is halved its K.D.$_{50c}$ increases by +1·75 minutes (cf+1·15 minutes of gammexane), and therefore this technique should be more accurate for the estimation of p.p. DDT than for gammexane.

(1180)   G

The p.p. content of a solution (containing by chemical analysis 3 per cent. p.p. DDT and 3 per cent. other hydrolysable chlorides, which probably consisted mainly of the ortho form of DDT), was estimated by this technique and the two values calculated were 1·5 and 2·0 per cent. p.p. DDT. There seemed no reason why such low values should be obtained and as later work also suggests (p. 427) it is possible that the oily impurities in DDT have an inhibitory effect upon the action of the p.p. compound.

Pyrethrum.

A number of experiments have been made to determine the speed of K.D. of solutions of pyrethrins. Some of these results are given in Table VIII and fig. 8. It was found that the percentage K.D. (probit)/time curve was approximately a straight line but there was considerable divergence from this simple relationship in the early stages. It appeared that the K.D. effect produced by pyrethrins occurred in two distinct phases. Firstly, immediately after spraying, a relatively large number of flies was knocked down and then there was a pause during which the number of K.D. flies either remained constant or more frequently actually decreased as some of the flies recovered (fig. 8 inset). This was followed by a second phase in which the number of K.D. flies increased regularly according to the usual percentage K.D. (probit)/time curve fig. 8. This double effect was not due to the particular definition of K.D. used here because it was as apparent when the percentage K.D. was calculated
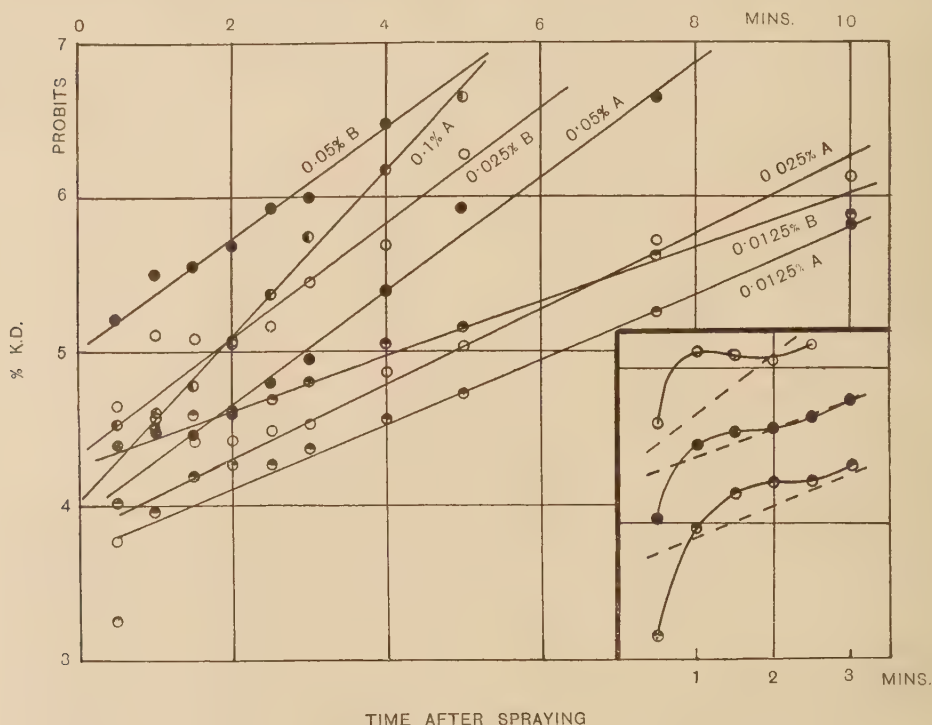
Fig. 8.—Speed of K.D. of *M. domestica* by various concentrations of pyrethrins. 0·1% = circles with left half closed; 0·05% = closed circles; 0·025% = open circles; 0·0125% = circles with upper half closed. A and B indicate Series A and B in Table VIII. Inset shows early stages of 0·0125% (closed circles) and 0·025% (open circles) of Series B and 0·0125% (half-closed circles) of Series A. Broken and continuous lines represent the expected and actual % K.D. respectively. Data from Table VIII.

from the number of flies actually on their backs (Series A) as when it was based upon those on the floor of the chamber (Series B).

TABLE VIII.

The speed of K.D. of *M. domestica* by varying concentrations of pyrethrins in petroleum distillate.

Time after Spraying in mins.	% K.D. by Pyrethrins						
	Series A				Series B		
	0·0125% (a)	0·025% (b)	0·05% (c)	0·1% (d)	0·0125% (e)	0·025% (f)	0·05% (g)
0·5	4	11	27	32	16	36	58
1·0	15	33	30	34	31	54	69
1·5	21	28	29	41	34	53	71
2·0	23	28	35	53	35	52	75
2·5	23	30	42	64	38	56	82
3·0	26	32	48	77	42	67	84
4·0	33	44	65	88	52	75	93
5·0	39	51	82	95	56	90	96
7·5	60	76	95	99	73	94	98
10·0	79	87	98	100	81	96	99
No. of Tests	3	3	3	2	4	4	4
No. of flies...	223	228	249	160	260	297	288
% Males ...	58	50	49	48	48	52	51
% Kill ...	28	41	53	84	31	41	61
K.D.$_{50c}$ in mins.	6·6	4·9	2·9	1·8	4·0	1·9	0·0
Expected K.D.$_{50c}$ in mins. from Formulae 7, 7a and 8	6·5 (6·8)	4·8 (4·8)	3·2 (2·8)	1·6	4·0	2·0	0·0
Difference in mins.	+0·1 −0·2	+0·1 +0·1	−0·3 +0·1	+0·2	0·0	−0·1	0·0

In Series A the % K.D. was assessed from the number of flies actually on their backs, whereas in Series B it was calculated from the number of flies on the floor of the chamber. Formulae 7, 7a and 8 have been fitted to columns a, b and c ; a, b, c and d ; and to e, f and g, respectively. Series A and B were carried out on separate days but the % kills show that the resistances of the two populations were very similar.

Once again there appeared to be a linear relationship between the median K.D. time of a solution and the logarithm of its concentration of insecticide (Table VIII). The following expressions have been fitted to the results of Series A and B :—

$$\text{Series A}—y= −5·426x−3·826 \quad ... \quad ... \quad 7$$

$$\text{Series B}—y= −6·645x−8·645 \quad ... \quad ... \quad 8$$

where y=K.D.$_{50c}$ in minutes.

x=logarithm of concentration of pyrethrins.

However, Series B lacks a value for 0·1 per cent. pyrethrins so that for comparison this value should be omitted from Series A in the calculation of its expression. If that is done the recalculated expression is :—

$$y = -6·645x - 11·445 \quad ... \quad ... \quad 7a$$

This is of interest because the expressions 7a and 8 have the same gradient, i.e. —6·645, which suggests that the change in definition of K.D. has merely resulted in shifting the $K.D._{50c}$ log. pyrethrin concentration line without altering its position relative to the two axes. In other words the two end-points (definitions of K.D.) represent different stages in the same process, so that not only are they each separately related to the pyrethrin concentration of the solution but they are also directly related to each other.* It follows therefore that the rather arbitrary definition of K.D. adopted here is a reasonably accurate measure of the effect of an insecticide solution on a population of *M. domestica.*

Some Results with DDT Deposits.

The Effect of the Nature of the Deposit on the Speed of K.D.

The experiments recorded on p. 413 in which sexed flies were exposed to films of pure p.p. DDT deposited from kerosene, were repeated two days later with another batch of flies, and the results were rather startling. In some samples the males were knocked down very much more quickly than the females whilst in others the males proved more resistant than the females (Table IX). Again, when the corresponding samples of the same sex are compared it is seen that the speed of K.D. of some of

TABLE IX.

Speed of K.D. of male and female *M. domestica* by a deposit of 20 mg. pure p.p. DDT/sq. ft.

Sample No.	$K.D._{50c}$ in minutes.					
	1st Test			2nd Test		
	Male	Female	Difference	Male	Female	Difference
1	21·1	25·4	+4·3	21·8	27·5	+5·7
2	21·5	25·7	+4·2	25·6	24·6	—1·0
3	23·5	28·7	+5·2	23·0	32·9	+9·9
4	29·0	30·4	+1·4	31·8	30·1	—1·7

First and second tests made on the 11th and 13th February, 1947, respectively.

TABLE X.

Comparison of two tests on the speed of K.D. of male and female *M. domestica* by a deposit of 20 mg. pure p.p. DDT/sq. ft.

Sample No.	$K.D._{50c}$ in minutes.					
	Males			Females		
	1st Test	2nd Test	Difference	1st Test	2nd Test	Difference
1	21·1	21·8	+0·7	25·4	27·5	+2·1
2	21·5	25·6	+4·1	25·7	24·6	—1·1
3	23·5	23·0	—0·5	28·7	32·9	+4·2
4	29·0	31·8	+2·8	30·4	30·1	—0·3

*In this example the simplest expression relating the two end points is :—$y = x + 2·8$; where x and y represent the $K.D._{50c}$ in mins. of solution k in Series A and B respectively.

the samples is approximately the same on both days whereas others show considerable difference (Table X). These anomalies are resolved when the comparison is made between the samples of flies exposed on the same set of panels (Table XI), from which it appears that whilst the speed of K.D. of flies exposed to one set of panels (100K/20C-2) has remained more or less constant that of the other set (100K/20C-3) has slowed down. It was also noticed that about half of the insecticide deposit on the panel* (100K/20C-2), which covered the observation window had been rubbed away, presumably by contact with the side panel over which it was drawn when observations were being made.

TABLE XI.

Effect of different sets of panels on the speeds of K.D. of male and female *M. domestica* by 20 mg. pure p.p. DDT/sq. ft.

Sex	Sample No.	1st Test	2nd Test	Difference
Panel Serial 100K/20C-2				
Male	1	21·1 mins.	21·8 mins.	+0·7 mins.
Male	3	23·5 ,,	23·0 ,,	−0·5 ,,
Female ...	2	25·7 ,,	24·6 ,,	−1·1 ,,
Female ...	4	30·4 ,,	30·1 ,,	−0·3 ,,
Mean Difference = −0·3 mins.				
Male	2	21·5 mins.	25·6 mins.	+4·1 mins.
Male	4	29·0 ,,	31·8 ,,	+2·8 ,,
Female ...	1	25·4 ,,	27·5 ,,	+2·1 ,,
Female ...	3	28·7 ,,	32·9 ,,	+4·2 ,,
Mean Difference = +3·3 mins.				

The simplest explanation of these results is that the flies used in the second series of tests were more resistant than those used in the first series by about 3·3 minutes and the grinding of the deposit on the upper panel in set 100K/20C-2 had provided the DDT in a more readily assimilable form, i.e. as a fine powder which could be ingested, and this had brought about the relatively quicker action of this set of panels.

Further evidence in support of this interpretation is to be found in the fact that deposits of ball milled DDT act more quickly than crystalline deposits of DDT from kerosene. On p. 406 experiments were made with four sets of panels coated with 20 mg. p.p. DDT/sq. ft. The median K.D. time calculated from four tests on each set of panels was 20·9±0·41 minutes whilst that of a set of panels treated with a deposit of 20 mg. p.p. DDT from kerosene was 25·1±0·86 minutes and the probability that such a difference could be due to random error is less than one chance in a hundred.

It had been observed that, after flies had been exposed to a deposit of ball milled DDT, the deposit was punctuated with numerous small round clear areas. Examination of these under a binocular dissecting microscope revealed that they were areas from which the deposit had been removed and each mark was of a similar size to the labella of a fly's proboscis and it was assumed that insecticide had been ingested by the flies and hence the insecticide was able to act both as a contact and stomach poison. Moreover, although crystalline deposits show the marks left by the flies' proboscises, careful examination showed no sign of any of the deposit having been removed. It seems that the crystals are too tightly adherent to the panels for the flies to be able to move them. This could be used as an argument in favour of the

*The panels were numbered and always placed in the same position in the testing chamber.

use of kerosene deposits of DDT when persistent residual action is required.   However, there are two other points which need to be considered in this connection.   Firstly, kerosene sprays penetrate the material sprayed more readily than water-based suspensions (Barlow & Hadaway, 1947).   Secondly, it has been shown that mosquitos can become activated by sublethal doses of DDT so that they avoid treated surfaces (Kennedy, 1947), and therefore it is important that in the period of contact before activation occurs the maximum amount of insecticide should have been absorbed. Of course, it is unlikely that this applies to mosquitos on account of their specialised mouthparts but the phenomenon of activation is probably much more widespread.

The relationship between the amount of p.p. DDT/sq. ft. and the speed of K.D.

Many experiments were carried out using panels coated with deposits of p.p. DDT ranging from 2·5 mg. to 40 mg./sq. ft. and a typical series of early results are discussed on p. 408, and represented in fig. 2.   However, after all the known sources of error, e.g. sex and intensity of illumination, had been minimised or eliminated, there was still no obvious correlation between the speed of K.D. and the density of the deposit.   For example, from three experiments the median K.D. times of a series of ball milled DDT deposits of 5, 10, 20, and 40 mg. p.p. DDT/sq. ft. were 23·3, 25·1, 24·2 and 21·0 minutes respectively.   It was usual to find that one of the lightest deposits showed a faster speed of K.D. than a much heavier deposit.   To investigate this point nine sets of panels were prepared having deposits ranging from 1·25 to 320 mg. p.p. DDT (ball milled)/sq. ft. and the results are set out in Table XII.

TABLE XII.

Median K.D. times in minutes of various deposits of p.p. DDT (ball milled).

Test No.	Concentration of p.p. DDT (ball milled)/sq. ft.								
	1·25	2·5	5	10	20	40	80	160	320
1	25·6	22·2	24·9	20·7	24·4	21·8	22·0	22·2	22·9
2	26·8	20·2	23·3	21·2	22·5	21·2	22·0	22·3	·24·8

From these results it is clear that the speed of K.D. is independent of the density of the DDT deposit, although it is possible that the high values obtained for the deposit of 1·25 mg./sq. ft. may represent a real difference.   This suggests that, on a non-absorptive surface, the speed of action is not accelerated by increasing the deposit and if therefore K.D. is affected by a certain concentration of p.p. DDT in the insects' body fluids, then the rate of penetration of DDT through the insect cuticle must be independent of the concentration of DDT with which it is in contact, providing that it is above a certain minimum.   From these experiments this minimum would appear to be about 2 mg./sq. ft.   This is the amount of deposit required to cover completely the panels with a continuous film of insecticide.   When smaller amounts of ball-milled DDT are applied it is impossible to obtain an uninterrupted film of insecticide by the method of application detailed on p. 406.

Alternatively the results can be explained without postulating the existence of a constant rate of entry of DDT into the insect body, by assuming that the small amount of DDT (Savit & others, 1946, suggest an L.D.$_{50}$ of 8–21 mg./kilogram) needed to bring about ultimate paralysis is very quickly absorbed from deposits heavier than 2 mg./sq. ft.   The absorbed DDT, it is suggested, acts by inhibiting an enzyme reaction and the later paralysis is due to the accumulation of toxic products. That is, the similarity between the K.D. speeds of 2·5 and 320 mg./p.p. DDT/sq. ft. is due to the similarity in the rate of accumulation of a toxic metabolite and not to a similarity in the internal concentration of DDT.   However, this explanation fails to account for the quicker K.D. when the insecticide is ingested (see p. 423).

The only factor which seemed to be related to the concentration of insecticide on the panels was that of the activity of the flies after they were K.D. Flies knocked down by light deposits of DDT remained relatively quiescent whilst those affected by heavy deposits had a period of frenzied activity.

Whatever the explanation of these results may be, they are of great interest when compared with those of Parkin and Green (1947). These workers deposited DDT from kerosene solutions on to samples of wall board, an absorptive material, and they recorded a much slower rate of K.D. of *M. domestica* by deposits of 40–320 mg. DDT (77 per cent. p.p.)/sq. ft. Moreover they obtained distinct differences between the speeds of action of the various deposits and had not reached the point at which an increase in deposit failed to produce a corresponding increase in the speed of K.D. This suggests that a deposit as heavy as 320 mg. p.p. DDT/sq. ft. on an absorptive surface does not act as quickly as 2·5 mg. p.p. DDT/sq. ft. on a non-absorptive material, and therefore that on an absorptive surface more than 99 per cent. of the insecticide has passed below the surface into the material where it is unable to make contact with insects which settle on the treated surface.

TABLE XIII.

Corrected median knock-down times in minutes of various concentrations of pure p.p. DDT deposited from kerosene solutions on to ordinary or paper-covered aluminium panels. Test 1 was carried out on 23rd March and Tests 2 and 3 on 25th March.

Deposit	Surface	Test No.			
		1	2	3	Mean
20 mg./sq. ft.	Aluminium	18 mins.	18 mins.	20 mins.	19 mins.
40 ,, ,,	Paper	45 ,,	40 ,,	57 ,,	46 ,,
20 ,, ,,	,,	43 ,,	49 ,,	65 ,,	53 ,,
10 ,, ,,	,,	130 ,,	175 ,,	—	158 ,,
5 ,, ,,	,,	200 ,,	—	—	200 ,,
2·5 ,, ,,	,,	—	216 ,,	—	216 ,,

This conclusion was further confirmed when tests were made on varying deposits of pure p.p. DDT which had been applied in petroleum distillate to both ordinary and paper-covered aluminium panels. The results are given in Table XIII and they show that the deposits put down on papered panels acted very much more slowly than those on the non-absorptive aluminium panels. Moreover the speed of action of the p.p. DDT deposits on the papered panels was directly related to their concentrations, so that the difference between the results given earlier in this paper and those of Parkin and Green (1947) is entirely due to the different surfaces treated. There is also a suggestion in this table that as a result of repeated tests an appreciable amount of the effective deposit on the papered panels is being removed and hence the speed of action is slowing down. Such an indication was never noticed with deposits on aluminium panels even on the 10th exposure to flies.

Comparison of the speed of action of deposits of DDT from 100 per cent. p.p. DDT with others from 80 per cent. p.p. DDT.

Three more tests were made on the ten sets of panels mentioned on p. 408. Of these sets, five were treated with 2·5, 5, 10, 20 and 40 mg. pure p.p. DDT/sq. ft. and the other five were treated with the same dosage of p.p. DDT deposited from a solution of technical DDT (80 per cent. p.p.). Observations were made simultaneously on the sets with the same deposit of p.p. DDT. The flies were conditioned overnight as usual, and consequently there was no correlation between the order of testing and the speed of K.D. such as was illustrated in fig. 2. The results of these tests are given in Table XIV from which it will be noticed that on only two occasions

D. S. KETTLE.

TABLE XIV.

Median K.D. times of deposits of p.p. DDT deposited from kerosene solutions of pure (P=100% p.p.) and technical (T=80% p.p.) DDT.

K.D.50c. in minutes.

Deposit p.p. DDT mg./sq. ft.	First Test			Second Test			Third Test		
	P	T	Difference	P	T	Difference	P	T	Difference
40	20·1	29·0	+8·9	18·7	24·9	+6·2	19·9	25·0	+5·1
20	23·8	30·0	+6·2	23·2	27·7	+4·5	23·9	29·9	+6·0
10	23·0	29·5	+6·5	25·2	25·1	−0·1	28·9	31·2	+2·3
5	21·8	25·6	+3·8	24·9	26·0	+1·1	32·2	29·8	−2·4
2·5	25·5	27·6	+2·1	24·2	28·9	+4·7	27·5	33·0	+5·5
Mean Difference in minutes per test ...	...	...	+5·5			+3·3			+3·3
all tests ...						+4·0			

out of fifteen was the speed of K.D. of a deposit of technical DDT as fast as or faster than an equivalent deposit of pure DDT. Since it has already been shown in the last section that under the conditions of experiment used here the speed of K.D. is not affected by the density of the deposit, it is permissible to treat these results as fifteen pairs of observations on pure and technical DDT. The mean median K.D. times and their standard errors for pure and technical DDT are $24 \cdot 2 \pm 0 \cdot 94$ minutes and $28 \cdot 2 \pm 0 \cdot 66$ minutes respectively and the probability that this difference may be due to random errors is less than one chance in one hundred. Therefore it is clear that a given deposit of p.p. DDT is more effective when applied as a solution of pure p.p. DDT than as one of technical DDT.

There are several possible explanations of this result. It has been shown by Parkin and Green (1947) that DDT deposits are more effective in a crystalline than in a supersaturated condition and so this result could be explained by the pure p.p. DDT deposits crystallising more readily than the technical DDT deposits. Although it was observed that the pure p.p. DDT deposits did crystallise more readily than the technical DDT deposits no panel was used until it was quite '' dry '' (see p. 406), that is, until crystallisation was complete. It seems, therefore, unlikely that the observed difference can be explained in this way, particularly since there was no detectable increase in efficiency of the technical DDT deposits after repeated exposures, which would be expected as the contact of the flies with the supersaturated solutions induced crystallisation.

McIntosh (1946) showed that the efficiency of a DDT suspension varies with the size and shape of the crystals, and he found that long needle-like crystals were the most effective. This is an unlikely explanation of the present result because from inspection (no measurements were made) it seemed that the crystals deposited from technical DDT were longer than those from pure p.p. DDT.

There are two other possible explanations. Firstly, the impurities in the technical DDT could, by mechanically covering up some of the p.p. DDT, lead to an increase in the untreated surface area. Then the cause of the slowness of the K.D. would be due to incomplete coverage, similar to that shown by $1 \cdot 25$ mg. p.p. DDT (ball milled)/ sq. ft. (see p. 424).

The second explanation is that the impurities in the technical DDT are antagonistic to the para para compound and inhibit its action. There is some support for this view in the low value of the p.p. content, calculated from its biological effect, given by a mixture of 3 per cent. p.p. DDT and 3 per cent. impurities (see p. 420).

Summary.

This paper is concerned firstly with the standardisation of a technique for the estimation of the speed of action of insecticidal sprays and deposits on adult house-flies (*Musca domestica*), as indicated by their speed of knock down, and secondly with the application of this technique to the assessment of the biological efficiency of insecticidal solutions. The main conclusions are :—

The speeds of action of both sprays and deposits are greatly affected by the duration and intensity of illumination to which the flies have been exposed before treatment and by the ratio of the sexes in the treated population. The first factor was eliminated by conditioning the flies to an intensity of illumination of 20 foot-candles overnight, i.e. at least 17 hours, and the second was minimised by estimating the median knock down time for 50c per cent. instead of 50 per cent. where $50c = 0 \cdot 67x + 0 \cdot 33y$, and x and y represent the percentages of males and females respectively in the treated population. This relationship appeared to be independent of the technique as it was the same for 0·05 per cent. gammexane in petroleum distillate used as a fly spray and for deposits of pure p.p. DDT put down from petroleum distillate. The experimental evidence, on which this correction is founded, is set out in full.

It was found that there was a separate straight line relationship between the percentage knock down in terms of probits and the time after spraying (or the time of exposure) in minutes for male and female *M. domestica*. It follows therefore that such a relationship is not true for a population of mixed sexes. The method of calculating the corrected median knock down time (K.D.$_{50c}$) is detailed.

The speed of knock down of solutions of pyrethrins, the gamma isomer of BHC and the para para isomer of DDT were proportional to the concentration of the insecticide in the solution. This conclusion has been applied to the estimation of gammexane in solutions of unknown strength and examples are given in which the calculated and actual values are compared and some conception is obtained of the accuracy of the method.

Experiments with DDT deposits on aluminium plates showed that :—

(*a*) The speed of action was independent of the deposit providing that this was above 2 mg. p.p. DDT/sq. ft. Although when the deposit was applied to paper-covered panels the speed of action varied with the magnitude of the deposit.

(*b*) A deposit of p.p. DDT from a solution of pure (100 per cent.) p.p. DDT acted more quickly than an equal p.p. DDT deposit from a solution of technical (80 per cent.) p.p. DDT.

(*c*) The action of a deposit was more rapid when the deposit was in a form which could act both as a stomach and contact poison and hence the ball milled technical DDT applied as a suspension acted more quickly than an equivalent deposit of technical DDT from petroleum distillate because the crystals of the latter were too tightly adherent to the panel for the flies to ingest them.

The significance of these results is discussed under the appropriate section.

Acknowledgements.

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STUDIES ON THE TOXICITY OF INSECTICIDE FILMS.*
III.—EFFECT OF RELATIVE HUMIDITY ON THE TOXICITY OF FILMS.

By S. PRADHAN.

Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts.

(Plate VII.)

So far little attention has been paid or significance given to the rôle of humidity in experiments on contact insecticides. Hence in the beginning no attempt was made to control humidity. As explained in the second part of this paper (Pradhan, 1949b), however, inconsistencies in the results of different experiments necessitated the investigation of the humidity effect and a preliminary experiment was carried out. This experiment showed that the effect of humidity was of importance. The problem was therefore followed up.

Technique of Humidity Control.

The general technique and material have already been described (Pradhan, 1949a) and only a short note on humidity control is given below. Owing to the conditions prevailing after the war, devices for the control of humidity were not readily available. The use of ordinary desiccators with supersaturated salt solutions involved the employment of more space than was available for carrying out a considerable number of replicated experiments, in which the effects of two or three humidities at several concentrations of insecticide were to be simultaneously studied. An effort was made, therefore, to improvise more convenient means. After some preliminary trials, a set-up illustrated in Plate VII, *a*, *b*, was decided upon. This consisted of one petri dish of about 9·3 cm. external diameter and two about 9·8 cm. internal diameter. The smaller petri dish is placed in one of the larger ones and in the annular space is put a small quantity of a saturated solution, with solid phase in reserve, of a suitable salt. The other petri dish is used as a cover, the joint between the edges of two larger petri dishes being rendered airtight by means of an adhesive cellulose tape (T) which also helps to hold the two dishes tightly together. This arrangement affords the required humidity, depending upon the salt used, within the space enclosed by the two larger petri dishes. The space provided by the inner smaller petri dish is used for housing the insects on the insecticide film. The humidity within this closed space was tested several times with the help of a dial paper hygrometer. Within half an hour or so after putting the tape round the edge, the humidity adjusted itself to within 10 per cent. of the expected value and within a few hours approximated the expected humidities.

In dealing with humidity in these experiments it is the relative humidity and not the saturation deficiency that has been compared. The main reason for this decision was that it was easier to select a few salts such as calcium chloride and sodium dichromate, that maintained humidity within a comparatively narrow range even when the temperature is changed within such limits as 56° to 90°F. Thus, $CaCl_2.6H_2O$ is known to maintain 38 per cent., 35 per cent., 32·3 per cent. and 31 per cent. R.H. at 10°, 18·5°, 20° and 24·5°C. respectively. Consequently, while studying the effect of temperatures from 90° to 56°F. the same salt could be used, e.g. sodium dichromate for maintaining relative humidity within a narrow range of 48 to 56 per cent. In order to maintain the saturation deficiency within such a narrow range it would have been necessary to use different salts or different strengths of solution of the same salt at different temperatures. This variation in salt or in its

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concentration was regarded as inadvisable, especially in the preliminary stages of the investigation on the temperature effect. It must, however, be admitted that sufficient consideration has not been devoted to this point since the aim of the experiments at this stage was to see if there was *any* effect of humidity on the toxicity of insecticide films.

Effect of Relative Humidity on the Toxicity of DDT Films to *Tribolium castaneum* Adults.

Experiment I.

Four different humidities were tested at four different temperatures at one concentration of the insecticide. The chemicals used for maintaining different humidities were :—

1. H_2SO_4 (98 per cent. concentration) for maintaining very dry conditions :—When tested with an Edney paper hygrometer the humidity showed much less than 20 per cent. but the true value could not be measured as this instrument was not graduated below 20 per cent. and probably the published values are more accurate for the conditions prevailing.

2. $CaCl_2.6H_2O$—Saturated solution with solid phase in reserve :—Theoretically, this should give 35 per cent. R.H. at 18·5°C. and 31 per cent. R.H. at 24·5°C. ; actually, the paper hygrometer showed 31 per cent. at 80°F. (21·7°C.).

3. $Ca(NO_3)_2.4H_2O$—Saturated solution with solid phase in reserve :—Theoretically, it should have 56 per cent. at 18·5°C. and 51 per cent. at 24·5°C., but in practice it was found to maintain 46 per cent. R.H. at 80°F.

4. $BaCl_2.2H_2O$—Saturated solution with solid phase in reserve :—Theoretically, this should maintain 88 per cent. R.H. at 24·5°C. (76°F.). It was found to have 84 per cent. R.H. at 80°F.

10/1/47. Forty-eight filter papers were sprayed as usual with benzene-water emulsion containing 0·1317 per cent. DDT and kept in C.T. cabinet. Twelve constant humidity chambers were prepared for each humidity using the above mentioned chemicals.

11/1/47. The films were transferred to C.H. chambers and 15 adults of *T. castaneum* were enclosed within glass rings on each film. Three C.H. chambers of each humidity containing insects and films were kept at each of the four temperatures (90°F., 80°F., 70°F. and 56–58°F.).

14/1/47. About 63 hours after enclosing the insects on the film, each batch was inspected on the warm C.T. plate.

TABLE I.

Experiment I.—Effect of temperature and humidity on percentage kill (dead, moribund and badly affected) in *T. castaneum* adults confined over film of DDT.

Chemical	Relative Humidity	Temperature			
		90°F.	80°F.	70°F.	56 to 58°F.
98% H_2SO_4	practically zero	96%	13·6%	8·7%	2·3%
$CaCl_2. 6H_2O$	about 30%	89·6%	12·2%	6·8%	9%
$Ca(NO_3)_2. 4H_2O$	about 50%	94%	14·6%	31·1%	0%
$BaCl_2. 2H_2O$	about 80%	98%	40%	61·7%	60%

The results are given in Table I. Although there is no clear-cut effect of humidity changes in the lower ranges, the highest humidity tested showed higher toxicity at all the four temperatures. The gradation of toxic effect under different humidity conditions was, however, quite obvious from the different amount of regurgitation which stained the films (Pl. VII, *c*) to different degrees. Final conclusions could not be drawn from these results as only one concentration was tried and at that concentration both the highest temperature (90°F.) and highest humidity (80 per cent. R.H.) appear to have acted as limiting factors, one masking the effect of the other. Thus humidity does not seem to play any significant rôle at 90°F. Similarly, temperature does not show any differential effect at about 80 per cent. R.H. For lower temperatures and humidities the concentration (0·1317 per cent. DDT) was

obviously too low to give any clear-cut gradation and so it was decided to test the humidity effect simultaneously at several concentrations.

Experiment II.

The same four humidities were tested at eight different concentrations of DDT. The same four chemicals were used for maintaining constant humidities. The temperature was maintained at 80°F.

24/2/47. Twelve filter papers were sprayed with each of the concentrations of DDT in benzene water emulsion and kept in C.T. cabinet for drying.

25/2/47. Insects were enclosed on control filter paper and films of concentrations 1 to 4. They were kept in C.T. cabinet at 80°F.

26/2/47. Insects were enclosed on concentrations 5 to 8 and kept at 80°F.

28/2/47. Inspection of insects on films of concentrations 1 to 4.

1/3/47. Inspection of insects on films of concentrations 5 to 8 and control.

TABLE II.

Experiment II.—Effect of humidity on the toxicity of DDT films to *T. castaneum* adults : continuous contact.

Concentration gm./100 cc.	Percentage of insects dead, moribund and badly affected			
	A practically 0% R.H.	B about 30% R.H.	C about 50% R.H.	D about 84% R.H.
Control	(2·2)	(2·8)	0	(4·4)
0·039	0·2	1·4	0·0	90·7
0·058	0·0	40·5	35·6	92·9
0·088	13	42·8	38·1	100
0·132	75·1	84·0	87·2	96·5
0·197	72·8	90·6	79·3*	100
0·296	76	91·0	77·8	100
0·444	100*	97·7	97·7	100
0·666	97·3	100	100	100

40–45 insects used for each test except where marked* the number was approximately 30.
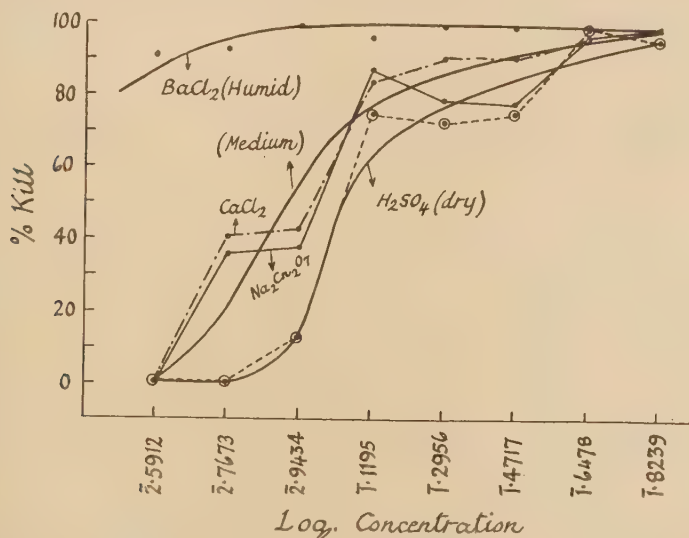
Fig. 1.—Effect of humidity on the toxicity of DDT films to *T. castaneum* adults : continuous contact at *four* humidities.

The data are given in Table II and fig. 1. It is clear that the lowest humidity maintained by 98 per cent. sulphuric acid showed least toxicity whereas the highest humidity maintained by barium chloride solution showed the highest toxicity. At intermediate humidities the toxicity was also intermediate, but there appears to be no significant difference in toxicity due to humidity changes in the intermediate zone.

Experiment III.

In this experiment the insects were confined on DDT films at the same humidity (R.H. 40 per cent.) for 24 hours and then transferred to a glass surface for reaction at four different humidities. If increased toxicity were due to greater " pick-up " then this experiment should not show higher toxicity at the higher humidities since insects had been kept in contact with the insecticide at the same humidity and temperature. The same constant humidity chambers were used as before.

10/3/47. Nine concentrations of DDT in benzene-water emulsion were prepared and 12 filter papers were sprayed with each concentration and kept in the C.T. cabinet at 80°F. The relative humidity in the cabinet was about 40 per cent.

11/3/47 (at 3.30 p.m.). Insects were enclosed on the films of concentrations 1 to 5 and on the control filter papers. The films were not removed from the dishes in which they were sprayed and the insects were enclosed on them within truncated glass cones and put back in the C.T. cabinet at 80°F. and 40 per cent. R.H. Thus all insects were kept in contact with the film at the same humidity and temperature.

12/3/47. (1). At 12 noon insects were also enclosed on concentrations 6 to 9 in the same way as on other concentrations.

  (2). At 3.30 p.m. the insects on concentrations 1 to 5 and control which had been in contact with the films for about 24 hours were transferred to glass surfaces in constant humidity chambers. Three batches from each concentration were transferred to separate C.H. chambers at each of 4 humidities and kept in C.T. cabinet at 70°F.

13/3/47. Insects from concentrations 6 to 9 were transferred to glass surfaces at different humidities and kept at 70°F.

15/3/47. Inspection on warm plate of insects on concentrations 1 to 5.

16/3/47. Inspection of insects on concentrations 6 to 7 and control. Concentrations 8 and 9 were not inspected.

TABLE III.

Experiment III.—Effect of humidity on the toxicity of DDT films to *T. castaneum* adults : contact at one humidity (40 per cent. R.H. and 80°F.) and reaction at *four* humidities (A, B, C and D and 70°F.).

Concentration gm./100 cc.	Percentage (dead, moribund and badly affected).			
	A	B	C	D
	practically zero	about 30% R.H.	about 50% R.H.	about 84% R.H.
Control	0	0	0	0
0·039	20	31†	55·6	76·2
0·058	28·9	56·6	81	84·8
0·088	52·2	88·9	97·7	91·3
0·132	93	100	100	100
0·197	88·5*	96·7†	81·6	100†
0·296	91	97·7	97·7	88·3
0·444	93	100	91·3	100

38–45 insects used for each test except where marked * where 26 and † where 30 were used.
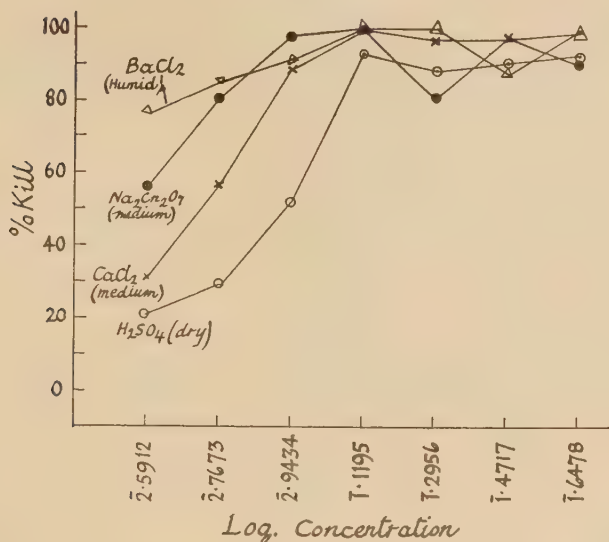
Fig. 2.—Effect of humidity on the toxicity of DDT films to *T. castaneum* adults : contact for 24 hours at 40 per cent. R.H. and reaction at four humidities : temperature 80°F. during contact and 70°F. during reaction.

The results are given in Table III and fig. 2. It is clear that on the whole this experiment also shows higher toxicity at higher humidity. This conclusion is based on the total of dead, moribund and badly affected insects. It may, however, be noted that the number of actually dead insects alone did not show any consistent result. There appears, however, to be no doubt about the ultimate toxicity being higher at higher humidities although the actual time of death appears to be controlled by a number of factors. On the whole a higher susceptibility was shown at all concentrations than would be expected from the results of the previous experiments. This fact becomes clear when curve marked ($Na_2Cr_2O_7$) in fig. 2 is compared with curve marked (70°F.) in fig. 6 of Pradhan (1949b, p. 249) which illustrates the susceptibility of *T. castaneum* when exposed to a film at 80°F. and allowed to react at 70°F. and 50 per cent. R.H. The probable cause for this higher susceptibility will be discussed later.

Experiment IV.

In view of the fact stated above, it was decided to repeat the third experiment with the same films and with both exposure and reaction at the same temperature (80°F.), instead of exposure at 80°F. and reaction at 70°F. as in that experiment, in order to lessen the toxic effect at all concentrations.

18/3/47.   Insects were enclosed on control and films of concentrations of Nos. 1 to 5 and kept as in Experiment III in the C.T. cabinet at 80°F. The relative humidity in the C.T. cabinet remained between 28 and 32 per cent. R.H.

19/3/47.   The above insects were transferred to glass surfaces in C.H. chambers and kept at four different humidities and at 80°F. instead of 70°F. as in the third experiment. More batches of insects were enclosed on films of concentrations Nos. 6 to 9.

20/3/47.   Insects from concentrations Nos. 6 to 9 were transferred to glass surfaces in C.H. chambers and kept at 80°F.

22/3/47.   The inspection of insects on concentrations Nos. 1 to 5.

23/3/47.   The inspection of insects on concentrations Nos. 6 to 9 and control.

           H

TABLE IV.

Experiment IV.—Effect of humidity on the toxicity of DDT films to *T. castaneum* adults : contact at R.H. 28 to 32 per cent. and 80°F. and reaction at four humidities (A, B, C and D at 80°F.).

Concentration gm./100 cc.	Percentage (dead, moribund and badly affected).			
	A	B	C	D
	0% R.H.	30% R.H.	50% R.H.	84% R.H.
Control	(2·2)	(4·5)	(2·3)	(0)
0·039	2·6	25·7	19	59·1
0·058	9·1	30·2	22	64·4
0·088	48·9	61·9	86	90·7
0·132	44	95·3	93·3	97·7
0·197	31·8	40·5	61·9	86·0
0·296	64·4	65	75·4	74·5
0·444	84	96·4	93·1	95·5
0·666	95·5	100	100	100
1·000	100	100	100	100

42–46 insects used for each test.
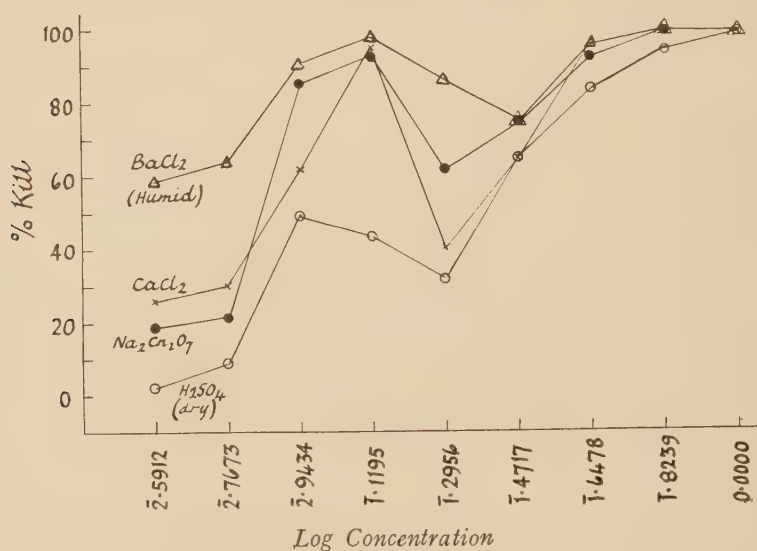
Fig. 3.—Effect of humidity on the toxicity of DDT films to *T. castaneum* adults ; contact at 30 per cent. R.H. and reaction at four humidities : temperature 80°F. throughout.

The results are given in Table IV and fig. 3. Despite some irregularity in the figures it is clear that this experiment confirms those of the last. Also the expectation of lower susceptibility in case of reaction at 80°F. instead of at 70°F. has been fulfilled.

Experiment V.

In this experiment the insects were kept in contact with the film at four different humidities for about 24 hours and then transferred to a glass surface and kept at the same humidity to react. The same films were used as in experiments III and IV.

25/3/47. Films kept in different C.H. chambers and insects enclosed on films of concentrations 1 to 5 and control.

26/3/47. The above-mentioned insects from concentrations 1 to 5 were transferred to glass surfaces in open petri dishes and kept in C.T. cabinet at 80°F. More batches of insects were enclosed on films in different C.H. chambers on concentrations Nos. 6 to 9.

27/3/47. Insects from concentrations 6 to 9 were transferred to open petri dishes and kept in the C.T. cabinet at 80°F.

29/3/47. Inspection of insects from concentrations Nos. 1 to 5.

30/3/47. Inspection of insects from concentrations Nos. 6 to 9 and control.

The data are given in Table V. It is clear that the lower humidities have not shown any clear-cut differential effect, but despite irregularities the highest humidity has shown a tendency to give the highest toxicities.

TABLE V.

Experiment V.—Effect of humidity on the toxicity of DDT films to *T. castaneum* adults : contact at four humidities (A, B, C and D at 80°F.) and reaction at one humidity and 80°F.

Concentration gm./100 cc.	Percentage (dead, moribund and badly affected)			
	A 0% R.H.	B 30% R.H.	C 50% R.H.	D 86% R.H.
Control	0	0	0	0
0·039	0	0	0	2·2
0·058	0	0 *	0	47·8
0·088	0	0	2·4	42·2
0·132	2·3	0	23	23
0·197	18·2	13·6	2·2	60
0·296	2·2	11·1	10·9	31·1
0·444	15	43·5	35·6	73·9
0·666	73·9	66·0	68·9	73·3
1·000	100	100	100	100

40–45 insects used for each test except where marked* in which 29 were used.

Discussion.

All the five experiments on the humidity effect show that at higher humidities the toxic effect of DDT films to *T. castaneum* is much higher. A comparison of experiment V with experiments III and IV shows that humidity is more effective during reaction than during exposure. It is naturally difficult to prove this statement completely because reaction cannot be inhibited during the exposure period. Judging, however, from the condition of the insects at the time of transference to glass surfaces it appeared that in experiment V even the higher toxicity observed in the case of those exposed at the higher humidity may not be due to actual exposure at higher humidity, but due to the reaction at that humidity during the exposure period itself.

Besides the humidity effect another interesting and important fact appears to have emerged from these experiments. In both experiments III and IV susceptibility was greater than was to be expected on the basis of previous experiments. The following is the most feasible explanation for this increase. The insects (*T. castaneum*) used in all previous experiments were reared and kept continuously at 80°F. until they were taken out for any particular experiment. But for a few weeks immediately previous to these two experiments (III and IV) there was a very cold spell of weather coupled with the fact that all heating arrangements were stopped owing to fuel shortage and consequently the insects had to be kept at ordinary unheated room-temperature. Such conditions probably increased their susceptibility considerably. This explanation is based on the results of Potter and Gillham (1946) who obtained results which

gave some indication that if the insects were kept in a cold environment previous to spraying they showed a higher susceptibility than if kept in a warm one.

Effect of Humidity on the Toxicity of DDT Films to *Plutella maculipennis* Larvae.

Experiment VI.

In order to test the conclusions arrived at with *Tribolium castaneum,* the following experiment was carried out with larvae of *Plutella maculipennis* as the test insect and DDT films made on circles of bolting silk instead of filter paper. Two humidities were tried : (*a*) practically zero per cent. R.H. and (*b*) about 84 per cent. R.H.

8/4/47. Twelve circles of bolting silk were sprayed with each concentration and kept at 80°F. for drying.

10/4/47. Six circles of each concentration were converted into cones, thus preparing six cages sprayed with each concentration. Three of these cages were kept in C.H. chambers with practically zero per cent. R.H. and the other three in C.H. chambers with about 84 per cent. R.H. Ten larvae of *P. maculipennis* were enclosed in each cage between 5 and 7 p.m. All cages were kept in C.T. cabinet at 80°F.

TABLE VI.

Experiment VI.—Effect of humidity on toxicity of DDT films to larvae of *P. maculipennis* : continuous contact.

Concentration gm./100 cc.	Percentage (dead, moribund and badly affected)	
	0% R.H.	84% R.H.
Control	(0)	(0)
0·039	60	24·1
0·058	82·8	30
0·088	83·9	32·1
0·132	96·7	58·1
0·197	92·6	43·3

28–31 insects used for each test.
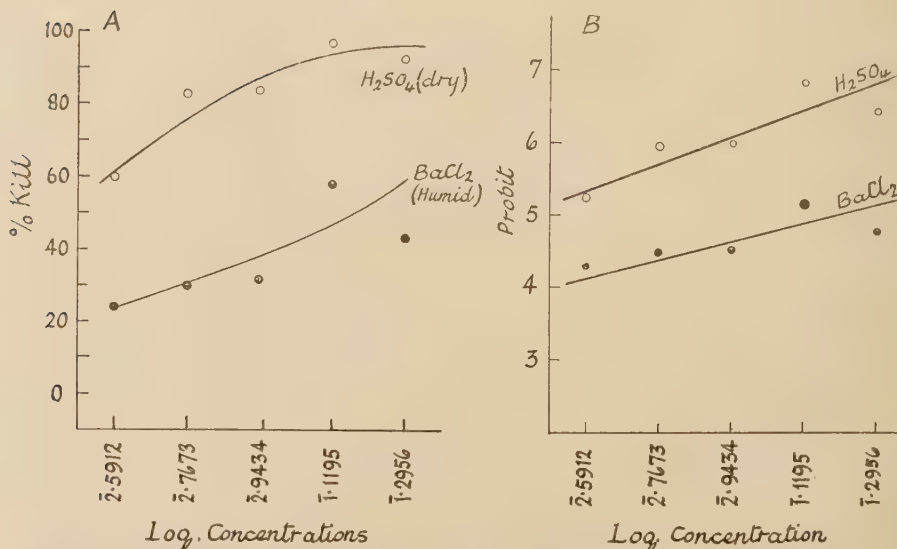
Fig. 4.—Effect of humidity on the toxicity of DDT films to larvae of *Plutella maculipennis* : continuous contact at two humidities.

The percentage of dead, moribund or badly affected larvae is given in Table VI and fig. 4. The results are fairly clear-cut. There has been much higher mortality under dry conditions than under moist conditions. Comparison of dead larvae taken alone shows a similar and, if anything, more marked difference. Thus the effect of humidity on the toxicity of DDT films to larvae of *P. maculipennis* proved diametrically opposite to that observed in the case of *T. castaneum*. In view of this complete reversal the experiment was repeated.

Experiment VII.

DDT films were made and the experiment carried through as in experiment VI.

TABLE VII.

Experiment VII.—Effect of humidity on the toxicity of DDT films to larvae of *P. maculipennis* : continuous contact : same as in Table VI except that the larvae were younger in this experiment.

Concentration gm./100 cc.	Percentage dead	
	0% R.H.	84% R.H.
Control	(0)	(0)
0·039	76	52
0·058	73	84
0·088	93	76
0·132	86	70
0·197	100	80

27–30 insects used for each test.

In this experiment the larvae proved to be much more susceptible than in experiment VI and this was obviously due to the fact that they were younger. Most of the larvae at both the humidities were either badly affected, moribund or dead. Hence a comparison of dead larvae alone is given in Table VII.

Except in concentration ·058 per cent., the mortality has been higher under dry conditions than under humid conditions thus tending to confirm the results of the previous experiment.

Effect of Humidity on the Toxicity of 3 : 5 dinitro-o-cresol Films to *T. castaneum* **Adults.**

The experiments with DDT films having demonstrated the effect of temperature and humidity on film toxicity, it was decided to ascertain if these effects were also true in case of other insecticides. Nicotine having presented certain difficulties, 3 : 5-dinitro-o-cresol (DNC) was finally selected as the second insecticide to be studied. Preliminary trials were made to fix the range of concentrations suitable for carrying out critical experiments on effects of temperature and humidity. These preliminary trials were carried out in the C.T. cabinet at 80°F. but no effort was made to record or control humidity. A range of concentrations of 0·17 per cent. to 0·8 per cent. wt/vol. was decided upon and an experiment with eight concentrations at regular intervals within this range was carried out in order to find out the effect of two temperatures (80°F. in C.T. chamber and 60°F. in basement). The humidity was maintained at about 50 per cent. R.H. by the employment of C.H. chambers, the humidities of which were controlled by the use of solutions of sodium bichromate. In this experiment there was no appreciable mortality or toxic effect even after several days continuous contact with the DNC film, although the same insect (adults of *T. castaneum*) was used in this experiment as in the preliminary trials. This result was surprising but, as the humidity had been maintained at a constant level, it

was decided to test the effects of varying this factor. Two humidities were tried : (*a*) about 50 per cent. R.H. as in the preliminary experiment, and (*b*) about 84 per cent. R.H.

Experiment VIII.

16/6/47. Seven concentrations of DNC in benzene-water emulsion with 0·05 per cent· C.H.D.S. (cyclohexylaminedodecylsulphate) as adjuvant were prepared and nine filter papers were sprayed with each concentration and kept in a C.T. cabinet (80°F.) to dry ; 5 cc. of the emulsion were used for spraying each filter paper.

17/6/47. Six filter papers of each concentration were kept in the sodium bichromate-controlled C.H. chambers (50 per cent. R.H.) and three in those controlled by barium chloride solution (84 per cent. R.H.).

18/6/47. Between 12 noon and 4 p.m., 15 adults of *T. castaneum* were enclosed on each filter paper. Those in C.H. chambers (84 per cent. R.H.) and three of those in C.H. chambers of 50 per cent. R.H. were kept in C.T. cabinet at 80°F. and the remaining three in C.H. chambers of 50 per cent. R.H. were kept in the basement at about 60°F.

19/6/47. A casual inspection was made without removing the insects to a warm plate and it was observed that practically all the insects in 84 per cent. R.H. were dead in highest 4 concentrations but none had died at 50 per cent. R.H.

21/6/47. Inspection was carried out in the usual way.

TABLE VIII.

Experiment VIII.—Effect of temperature and humidity on the toxicity of DNC films to *T. castaneum* adults.

	Percentage (dead, moribund and badly affected)		
Temperature ... R. Humidity ...	80°F. 84%	80°F. 50%	about 60°F. 50%
Concentration gm./100 cc.			
Control	0	0	0
0·168	78	0	0
0·209	100*	0	0
0·262	91	0	0
0·328	98	0	0
0·409	100	0	4
0·512	100	2	4
0·64	98	4	2

44–47 insects used for each test.
*30 insects used.

The results are given in Table VIII. The effect of humidity is too obvious to necessitate plotting or statistical analysis of the data. Thus in the case of DNC, the effect of humidity is similar to that shown by DDT, but, with DNC, humidity apparently plays a far stronger rôle.

The above effect was tested several times in small-scale trials with the same result.

Effect of Humidity on the Toxicity of DNC Films to *P. maculipennis* Larvae.

Only small-scale tests were carried out on the effect of humidity on the toxicity of DNC films to larvae of *P. maculipennis* but the result in each case was so clear-cut that the conclusion drawn can be taken to be fairly well established.

Experiment IX.

Four concentrations of DNC in benzene-water emulsion were prepared with 0·05 per cent. C.H.D.S. as adjuvant and four circles of bolting silk were sprayed with each concentration and kept in C.T. cabinet to dry.

6/6/47. Two circles sprayed with each concentration were converted into cones, thus completing two cages of each concentration. One of these cages was put in C.H. chamber at 84 per cent. R.H. and the other at approximately 0 per cent. R.H. and about 20 larvae of *P. maculipennis* were enclosed in each cage and kept in C.T. cabinet at 80°F.

TABLE IX.

Experiment IX.—Effect of humidity on the toxicity of DNC films to larvae of *P. maculipennis*.

Concentration gm./100 cc.	Percentage (dead, moribund and badly affected)	
	0% R.H.	84% R.H.
Control	0	0
0·168	0	25
0·262	5	90
0 328	0	100
0·64	0	100

17–20 insects used for each test.

The results are given in Table IX which shows that the DNC film was much more toxic under humid than under dry conditions. These results were subjected to a further test.

Experiment X.

17/6/47. Fresh films were prepared on 8 circles of bolting silk by spraying with 0·409 per cent. DNC emulsion.

18/6/47. Four circles were converted into cones, thus completing 4 cages, two of which were kept in C.H. chambers at 84 per cent. and the other two in C.H. chambers at zero per cent. relative humidity. Ten larvae of *P. maculipennis* were enclosed in each cage and kept at 80°F.

Inspection on the 19th revealed that all the insects had died in the C.H. chambers at 84 per cent. and none at zero per cent. relative humidity.

In order to see if there was any basic difference in the toxicity of these films they were interchanged and ten fresh larvae were enclosed on each.

Inspection on the 20th again revealed that all the larvae died on the films kept at 84 per cent. R.H. and none in the chambers at or about zero per cent. relative humidity.

Thus the differential toxicity was due to environment. In order to establish that the differential toxicity was due to differential humidity and not to any chemical effect of the barium chloride or sulphuric acid used for controlling it, these chemicals were replaced in fresh C.H. chambers by distilled water and anhydrous calcium chloride respectively. Ten fresh larvae were enclosed in each of these fresh C.H. chambers on the same film as was used in the foregoing tests. The inspection after about 24 hours showed again that all the larvae had died in the water chamber and none in calcium chloride chamber. The differential effects observed in these experiments were therefore shown to be due to the relative humidity of the respective environments.

Discussion and Review of Literature.

As in the case of the experiments described in the foregoing pages, a perusal of the literature does not help to deduce any general principle regarding the effect of humidity on the toxicity of insecticides. In some cases the rise in relative humidity has been found to increase the percentage kill and in others to decrease it. There are also cases in which changes in relative humidity have been found to have no appreciable effect on toxicity. All these cases are summarised in Table X.

All the observations given in this table, however, are not of equal reliability as some are results of planned investigation and others appear to be rather casual observations. For example, the range of humidity actually tested is not mentioned in each case, whereas it seems to be very important in certain cases at least. Hansens (1944) found that the toxicity of certain ovicidal powders to the body louse was generally high at relative humidities above 68 per cent., but that the results between 61 and 68 per cent. relative humidities were rather erratic while with humidities below 60 per cent. the toxic effects were not appreciable. Eddy (1947) confirmed Hansens' observations. He writes " In general the materials gave an excellent kill of eggs, at relative humidities of 80% or higher but little or no kill at 63% or lower ". In the experiments described in the foregoing pages, only the very high humidities (about 84 per cent.) have consistently exhibited a toxic effect, the changes in humidity in the intermediate zone producing comparatively much less effect. The conclusion of Mellanby (1936) that the rate of metabolism is governed by temperature alone and is not affected by humidity changes is of interest in this connection. He studied the metabolism of several species of insects but the humidities tested appear to range only between 0 and 60 per cent. The toxicity experiments described in this paper indicate that humidities above 60 per cent. may quite possibly bring about a change in insect metabolism.

The strength of the insecticide with which the humidity effect is tested is another factor of some importance. Steiner and Arnold (1943) found that weaker lead arsenate spray had no significant difference in effectiveness at different humidities, but the stronger spray increased in toxicity with higher humidities. Hansens (1944) found that 10 per cent. ovicidal powders " gave effective kills over a wider range of humidity than 1% powders ".

Different stages of the same insect may show different susceptibilities to poisons at different humidities. Thus Lindgren and Shepard (1932) found that with both chloropicrin and carbon disulphide the toxicity to adults of *T. confusum* was not affected by ordinary variations in relative humidity, but that dry air conditions reduced materially the effect of both these fumigants on eggs of the same species. On the other hand with methyl bromide Fisk and Shepard (1938) found the reverse to be true, i.e. with eggs of *T. confusum* the humidity effect was so little that the difference was of doubtful significance but with adults the toxicity definitely increased at higher humidity. It is, moreover, clear that the same stage of the same insect may show different humidity effects with different insecticides. A further instance is afforded by the present experiments with larvae of *P. maculipennis* whose percentage mortality decreases at higher humidity in the case of DDT films and increases at higher humidity in the case of DNC films.

Summary.

Experiments on the effect of changes in relative humidity on the toxicity of DDT- and DNC-films to adults of *Tribolium castaneum* and larvae of *Plutella maculipennis* are described. With *T. castaneum* adults the toxicity of both DDT and DNC films is increased at higher relative humidities, but with larvae of *P. maculipennis* the toxicity of DDT films decreases while that of DNC films increases at higher humidities. Like these experiments the perusal of the literature does not

TABLE X.   Summary of observations of various workers on the effect of humidity on the toxicity of insecticides.

Effect of increase in humidity on toxicity	Form in which the insecticide was applied	Test insects	Chemicals (Insecticide)	Author
Increased toxicity at higher humidities	Spray	Newly hatched codling moth larvae	Lead arsenate (stronger conc.) phenothiazine and nicotine	Steiner and Arnold (1943)
	Dust	Body louse eggs (*Pediculus humanus corporis*)	Ten different powders	Hansens (1944)
	,,	Body louse (presumably adults)	Several organic compounds	Eddy (1947)
	Film	Houseflies	DDT	Laug (1946) / Present observations
	,,	*T. castaneum* adults	DDT	,,
	,,	*T. castaneum* adults	DNC	,,
	,,	*P. maculipennis* larvae	DNC	,,
	Vapour	*T. confusum* eggs	Chloropicrin and carbon disulphide	Lindgren and Shepard (1932)
	,,	*T. confusum* eggs and pupae	Chloropicrin and carbon disulphide	Lindgren (1935)
	,,	*T. confusum*	Methyl bromide	Richardson and others (1943)
	,,	*T. confusum* adults	Methyl bromide	Fisk and Shepard (1938)
	Dip	*Ahasverus advena*	Standard derris insecticide	Craufurd-Benson (1938)
	Spray	*Eutettix tenellus*	Pyrethrum extract	Harries and others (1945)
Decreased toxicity at higher humidities	Dust	Different forest pests	Pyrethrum	Gösswald (1934)
	,,	*Calandra granaria*	Micronized felspar	Parkin (1944).
	Vapour	Red scale (*Aonidiella aurantii*)	HCN	Quayle and Rohrbaugh (1934) and Quayle (1934)
	,,	*T. confusum* adults	Methyl bromide	Fisk and Shepard (1938)
	Film	*P. maculipennis* larvae	DDT	Present observations
No effect of changes in humidity on toxicity	Spray / Spray mist	Newly hatched codling moth larvae / *Aedes aegypti* adults	Lead arsenate (weaker concentration) Pyrethrins	Steiner and Arnold (1943) / David (1946)
	Vapour	*Calandra oryzae* (presumably adults)	Chloropicrin	Bertrand and others (1919)
	,,	*C. granaria* (presumably adults)	Chloropicrin	Chapman and Johnson (1925)
	,,	*T. confusum* adults	Chloropicrin and carbon disulphide	Lindgren and Shepard (1932)
	,,	*T. confusum* adults and larvae	Chloropicrin and carbon disulphide	Lindgren (1935)
	,,	*T. confusum* eggs	Methyl bromide	Fisk and Shepard (1938)
	,,	*Phenacoccus gossypii* (mealybug)	Methyl bromide	Richardson and others (1943)
	,,	*Tetranychus bimaculatus* (red spider)	Methyl bromide	Richardson and others (1943)
	,,	Household insects	Tetrachloracetonitrile alone and plus acrylonitrile	Glass (1944)

help in deducing any general principle. However, it is possible to collect instances showing that besides the insect and the insecticide, there may be various factors like the *range* of humidity tested, the *strength* of the insecticide used, the *stage* of the insect, etc., which can affect the result of humidity changes.

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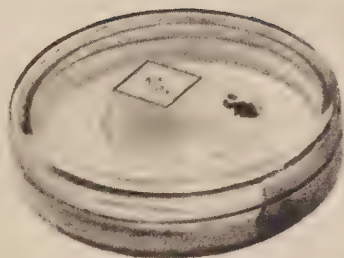
(a) Device for controlling relative humidity within a petri dish. For details see text. Within this device *T. castaneum* adults are confined over a clean filter paper within a glass ring. (Note that all the insects are huddled together, indicating that they have settled down normally.)
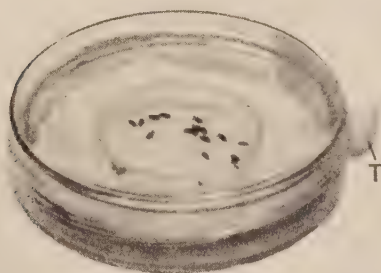
(b) The same device as above. Here *T. castaneum* adults are confined over a DDT film within a glass ring. (Note that the insects are scattered within the ring.) T = adhesive tape.
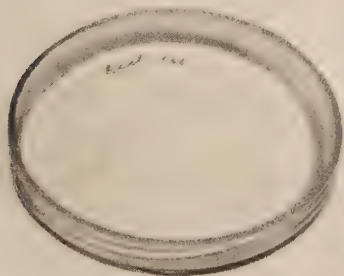
(c) Photograph of a DDT film over filter paper stained by regurgitation when *T. castaneum* adults were confined over it under humid conditions.

ON THE INTRODUCTION OF *SCOLIA RUFICORNIS*, F., INTO WESTERN SAMOA FOR THE CONTROL OF *ORYCTES RHINOCEROS*, L.

By H. W. SIMMONDS, O.B.E.
Late Govt. Entomologist, Fiji.

Oryctes rhinoceros, L., the Asiatic Rhinoceros beetle, was accidentally introduced into Western Samoa, apparently with rubber plants from Ceylon, sometime prior to September 1910. The results were disastrous to the coconut industry, and caused grave anxiety to coconut planters in the adjoining groups.

In April, 1912, F. P. Jepson, then Government Entomologist, Fiji, visited Samoa and reported on the position at that time. In the following year, Dr. K. Friedrichs made an extensive search for parasites of this pest in the Eastern tropics. He had just reached Madagascar when war broke out and brought his search to a close but he recommended that an attempt should be made to introduce *Scolia oryctophaga*, Coq., into Samoa from that country.

In 1933, the writer was seconded for three months to Western Samoa to report and advise on the general position at that date. In 1939, he undertook a mission to Java, Malaya, Mauritius and Madagascar in search of natural enemies and, failing anything new, to attempt to introduce *S. oryctophaga*. He was unable to find any true parasite of *O. rhinoceros*, but landed 210 adult female *S. oryctophaga* successfully in Samoa, although he had come to the conclusion that, in the absence of a definite cool season in Samoa, this species was not likely to succeed (Simmonds, 1941). He had, however, been informed that a Scoliid, labelled *S. carnifex*, Coq., in the Zanzibar Museum was recorded on the label as being a parasite of the Rhinoceros beetle. *S. carnifex* was present in Mauritius, and tests there had shown that it did not attack *Oryctes*, so an opportunity was taken of calling at Zanzibar to investigate the matter. It was then found that the Scoliid in the Museum was wrongly labelled, being a very different species subsequently identified by the Imperial Institute of Entomology as *Scolia ruficornis*, F. Tests showed that this species was indeed a parasite of the East African species of *Oryctes*.

After only partially successful tests on *O. rhinoceros* in Singapore, where the work was hindered by war conditions, the author advised that the indications were sufficiently encouraging to warrant a trial of this parasite. As he feared, *S. oryctophaga* failed to make good (not necessarily on account of the absence of a definite cool season, although the evidence points to this being the main factor controlling development in the pupal stage) and, in 1945, he was asked to undertake the introduction of *Scolia ruficornis* from Zanzibar. In this he was successful, some 465 adult females reaching Samoa in good order.

As this parasite had not actually been shown to be capable of breeding upon *O. rhinoceros*, despite the fact that tests had indicated that it should be able to do so, considerable anxiety was felt and the appearance of the next generation eagerly awaited. The chief doubt was as to whether the sting of this wasp would prove fatal to the Asiatic beetle. Six months after the release of the parasite, a report that one example had been seen and was very encouraging but, as no further reports were received, this was gradually discounted. In April, 1947, an American Entomologist, Mr. F. A. Bianchi, of the Experimental Station of the Hawaiian Sugar Planters' Association, made a careful search and failed to find any evidence of its presence, and further introductions were proposed.

On 8th February, 1949, however, a cable was received stating that 20 or 30 adults had been observed at 10 a.m. on the 6th, flying rapidly around a compost heap,

and on the 14th a specimen of one of these was received and proved to be a male of *S. ruficornis*. This brought three and a half years of uncertainty and anxiety to a most satisfactory conclusion. There seems little doubt that the slowness of the parasite in reappearing was due to the absence of any suitable concentration of host material in the right stage at the time of liberation, so that the parasites were compelled to scatter. This, with the long pupal period (about six months), would render the next generation very thinly distributed and mating precarious.

So far as one can prophesy, the future numbers and value of the parasite will largely depend upon the presence of sufficient honey-producing flowers at the right seasons of the year, but the provision of attractive breeding places for the beetle, of such a nature that the wasp would have no difficulty in reaching its host, is also desirable.

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APPENDIX.

With the concurrence of Mr. Simmonds, I wrote to Mr. C. E. Pemberton of the Experiment Station of the Hawaiian Sugar Planters' Association, as it was thought that he might have some further information on the recoveries of adult parasites, and I am indebted to him for the following most interesting details contained in an air letter dated the 12th September, 1949.—[ED.]

" Mr. L. J. Dumbleton of the Entomological Research Station, Nelson, New Zealand, has written me under date of 23rd August, 1949, that he has just returned from a visit to Samoa, where he made a study of the *Oryctes* situation. He reports that *Scolia ruficornis* is definitely established. However he was only able to find the adults in one locality. This was at a compost pit in a cocoa plantation about 20 miles from the place where the parasites were originally liberated. He stated that ' up to a dozen might be seen at any one time. These were mostly males with about 10 per cent. females '. He found no parasitized *Oryctes* grubs but dug up a single empty cocoon which he believed must have been made by the *Scolia*. His survey lasted two weeks and in no case was an adult parasite seen elsewhere.

" Some months ago I requested Mr. J. B. Menardi, Superintendent of our Samoan substation of this Experiment Station, to go to Western Samoa and investigate the *Scolia* situation. He did so this past summer and found *Scolia ruficornis* adults at the same compost pit discussed above ; but failed to see the parasite in any other locality.

" It is quite evident that the parasite is established ; but it is apparently uncommon excepting in the one locality."

STUDIES ON THE ACTION OF DDT ON ANOPHELINE MOSQUITOS AND HOUSE-FLIES.

By A. N. JOHNSTON, B.Sc.Agr.

Lecturer in Botany and Entomology, Hawkesbury Agricultural College, Richmond, N.S.W.

Studies on the toxicity of DDT on various stages of Anopheline mosquitos and house-flies were carried out by the writer while Officer Commanding No. 5 Australian Mobile Entomological Unit stationed at Cairns.

Material for the tests on mosquitos was provided from the nucleus colony of *Anopheles* (*Myzomyia*) *punctulatus punctulatus*, Dön., bred at the Medical Research Unit, Cairns, while house-flies were caught wild from rubbish dumps in the Cairns area.

The purpose of the work was to gather information on the physiological action of DDT on Anophelines and flies which could be applied to such field problems as larvicidal control, residual larvicidal action, residual control of adult flies and mosquitos.

The work was generally divided into the following four sub-groups and the experimental procedure is explained under each group.

A. Experimental toxicity studies on the larvae of *A. p. punctulatus*.

B. Action of DDT on the pupae of *A. p. punctulatus*.

C. Residual effect of DDT on the mortality of adult *A. p. punctulatus*.

D. Studies of residual action of DDT on house-flies.

A solution of 4 per cent. para para DDT in malariol was used for the work on mosquitos and 4 per cent. p.p.DDT in kerosene was applied for residual action of DDT on house-flies.

Group A.—Toxicity to Larvae.

The effect of DDT on larvae presented two problems. Firstly the effect of varying periods of exposure of larvae to a DDT oil film and secondly the reaction of larvae of different stages to DDT.

(1) *Knockdown of 4th stage larvae after various periods of exposure.*

A number of dishes of surface area of 1 sq. ft. were filled with water to a depth of 1 inch. One dish was treated with DDT at the rate of 2 qts./acre (227 gm. DDT acre or 4·3 mgm./sq. ft.) and 4th stage larvae were then pipetted into the dish and exposed for various periods of from one second to 15 minutes. Following exposure, the larvae were washed twice and placed in clean water. Twenty larvae were used for each exposure.

One set of larvae was removed after exposure for one second at the surface as it was noticed that, particularly in short exposures, larvae could sink to the bottom and remain without contacting the surface.

A control set and a set continuously under DDT were also maintained.

Mortality rates were taken by examining the dishes periodically and recording the number " dead (d) ", " pupated (p) ", or " emerged and dead (e and d) ".

The pupae recorded in the results emerged and died, except in the case of the control when all pupae emerged alive.

TABLE I.

Experiment started at 3.30 p.m. Mortality of 20 larvae following various periods of exposure.

Mortality after	1 sec. exp.	5 sec.	15 sec.	30 sec.	1 min.	5 min.	15 min.	1 sec. on surface	Continuous exposure	Control
3 hours (6.30 p.m.)	—	2	1	2	2	7	9	7	16	Nil
7 hours (10.30 p.m.) ...	1	3+1p.	3+1p.	3+1p.	4	8	15	9+1p.	20	3p.
17 hours (8.30 a.m.) ...	4	5+1p.	6+1p.	3+1p.	4	9	18	14+1p.		3p.
22 hours (1.30 p.m.) ...	4	6+1p.	6+1p.	3+1p.	10+2p.	16+1p.	20	16+1p.		9p.
24 hours (3.30 p.m.) ...	7+1p.	12+1p.	8+1p.	4+2p.	12+2p.	17+1p.	20	17+2p.		10p.
Percentage mortality after 24 hours	35	60	40	20	60	85	100	85	100	0

It would appear that the rate of mortality varies with the period of exposure to the DDT oil film. Low periods of exposure to DDT gave fairly satisfactory mortality results, and this links up with experience in the field where a complete film of DDT on an infested water surface is not required, and that low dosages give effective field control.

(2) *The reaction of different stages of larvae to a DDT oil film.*

Four sets of twenty larvae of each stage of development were prepared in dishes containing water similar to that described in (1). The age and development of the larvae were obtained from records of the Medical Research Unit and checked by microscopic examination.

Two of each set were treated with DDT at the rate of 1 qt. per acre (114 gm. DDT/acre) and the other two kept as controls. Mortality rates were then recorded.

TABLE II.

Action of DDT on different stages of Anopheline larvae.

Mortality after	T_1	T_1	T_2	T_2	T_3	T_3	T_4	T_4
¾ hour	2	2	—	1	2	—	3	2
1¼ hour	5	7	2	3	11	7	3	2
3¾ hours	14	17	6	9	16	14	11	9
24 hours	19	20	15	16	19	18	17	16
							+1pd	+2pd
Percentage mortality in 24 hours ...	95	100	75	80	95	90	90	90

$_1$= 1st stage larvae. $_2$= 2nd stage larvae. $_3$= 3rd stage larvae. $_4$= 4th stage larvae.
There was no mortality in any of the control sets.

No significant difference between the reaction of different stages is apparent ; the rate of mortality and the final mortality figures in all cases show a close relationship.

Group B.—Toxicity to Pupae.

Evidence was obtained that Anopheline pupae may be more sensitive to DDT than is generally believed. A very high death rate occurs ultimately but signs of poisoning develop more slowly than in the larvae, and many mosquitos do not die

TABLE III.

	A. punctulatus larvae		*A. punctulatus* pupae				*Aëdes vigilax* pupae
Dosage 5 per cent. DDT/acre ...	1 pint	2 pints	1 pint		2 pints		2 pints ?
Exposure	Continuous	Continuous	25 min.	Continuous	25 min.	Continuous	Continuous
No. treated ...	40	200	10	10	10	50	180
No. killed	36	190	—	8	5	48	165
No. emerged and died	—	—	10	2	5	2	15
Percentage mortality	90	95	100	100	100	100	100

until emergence.   The experiments showed that this is not due to the residual effect of DDT in the water on the emerging adults, because the pupae were removed from the treated water, washed twice and placed in clean water for emergence.   The results of a number of experiments are collated in Table III where comparison of the mortality of larvae and pupae of *A.* (*M.*) *punctulatus* and pupae of *Aëdes vigilax*, Skuse, is made under continuous exposure to DDT and 25 minutes' exposure to DDT followed by removal and washing.

The results in Table III have been confirmed by field tests ; they indicate that pupae are just as susceptible to DDT as larvae but that the slowness of action in the case of pupae may be due to anatomical features.

Group C.—Residual Effect on Mortality of Adults.

Experiments were designed to test the behaviour of adult Anophelines to the residual effect of DDT in order to obtain information that would be of value in the control of the adults in the field.

Two sets of experiments were carried out—the first in the laboratory and the second in the field.

Laboratory Trials.

Strips of two-ply wood treated with DDT at the rate of 100 mgms. per sq. ft. were placed in 1-in. tubes with Anophelines.   The mortality figures are based on ten replications.

Of 31 Anophelines, 28 were down and 2 dead after $\frac{3}{4}$ hour, the corresponding figures for $1\frac{3}{4}$ and $2\frac{1}{4}$ hours were 13 and 18, and 0 and 31 respectively.   Of 30 control mosquitos none were dead after $\frac{3}{4}$ hour, but one died after $1\frac{3}{4}$ hours.

A further experiment in which test tubes lined with leaves treated with DDT at the rate of 100 mgm. per sq. ft. was also carried out.   The mortality figures in this case are based on five replications.

TABLE IV.

	Test		Control	
No. of Anophelines	17		14	
Time	down	dead	down	dead
$\frac{3}{4}$ hour	2	—	0	—
$1\frac{1}{4}$,,	14	—	—	1
$1\frac{3}{4}$,,	12	4	—	1
$2\frac{1}{4}$,,	6	11	—	1
$2\frac{3}{4}$,,	—	17	1	1

In repeated tests, no differences were noted between smooth surfaced *Croton* leaves, waxy leaves of Bird Nest Fern or hairy leaves of *Coleus*, all treated at the rate of 50 mgms. per sq. ft.

The time required to absorb a lethal dose was examined by a similar technique, the insects being removed after the requisite exposure and the mortality recorded at various times up to six hours later.

TABLE V.

Duration of exposure ...	30 sec.	1 min.	5 min.	15 min.	30 min.
No. of Anophelines ...	6	6	6	6	6
Result after 2 hours ...	0	0	0	5d	5d
Result after 6 hours ...	1D	1D	4D	5D	2d+4D

d=down   D=dead

These results confirm previous observations made by Waterhouse and others that Anophelines require at least 15 minutes' exposure to acquire a lethal dose of DDT and that they die slowly even when exposure is continuous.

Field trials.

An area of open scrub was chosen at Cairns consisting of Paperbarks, Cycads, Eucalyptus, Lilies, Pandanas, Banksia and Acacia, lying at the edge of a mangrove swamp, in which large numbers of *A. (M.) farauti*, Lav., were breeding.   The area, 300 × 60 yds. in extent, was sprayed at the rate of 6 gals. per acre (2,724 gm. DDT/ acre).   The Anopheline population dropped immediately, but after nine days 25–50 per cent. of this drop had been made good. It is worthy of note that, in contrast to the feeble effect against Anophelines, the population of the vicious biting *Aëdes vigilax* was reduced immediately, and reinvasion did not occur until the 38th day after spraying.

Residual spraying would appear practicable only where the resting habits of Anophelines are well known.

Group D.—Action of DDT on House-flies.

Residual treatment.

An experiment was set up, using strips of plywood treated with DDT at 100 mgms. per sq. ft. in 1-in. test tubes, as described for the treatment of Anophelines in an earlier section.   After exposure, the flies (*Musca domestica*, L.) were removed and the progress of poisoning recorded every half hour.

TABLE VI.

Duration of exposure	5 secs.		30 secs.		1 min.		5 min.	
No. of flies used ...	8		19		23		28	
	down	dead	down	dead	down	dead	down	dead
After ½ hour	6	—	19	—	23	—	28	—
,, 1 ,,	8	—	19	—	19	4	8	20
,, 1½ ,,	6	2	10	9	16	7	5	23
,, 2 ,,	3	5	3	16	4	19	0	28
Percentage mortality at 2½ hours ...	63		84		82		100	

None of the 10 insects forming the control were " down " or " dead " at the end of 2½ hours.

It is clear from these results that the duration of exposure needed to acquire a lethal dose and the time required to kill are very much shorter for flies than mosquitos.

Distribution of DDT by poisoned flies.

The slow death and staggering gait of poisoned flies suggested that they might be capable of distributing DDT mechanically from treated to untreated areas. A series of tubes (*a*) was set up, as described above and a total of 103 flies placed in them. After 10 minutes, 46 of the flies were transferred to a second series (*b*) of five clean tubes containing untreated strips, where they were allowed to remain for two hours, the result at the end of that time being :—

Remaining in series (*a*) (treated)— 57 flies, 5 down 52 dead.

(*b*) (untreated)—46 flies, 12 down 34 dead.

The flies were removed from series (*b*) and 61 fresh flies were placed in these tubes. Deaths in this batch were 100 per cent.

Summary.

Experimental results indicated that the mortality rate of Anophelines depends on the time of exposure. No evidence of differential reaction between the various stages of larvae was found.

Definite and positive evidence of the action of DDT on mosquito pupae was obtained.

Laboratory trials indicated that mortality of adult Anophelines following contact with DDT is relatively slow, and the period of exposure required for a lethal dose is approximately 15 minutes.

Adult flies required a much shorter period of exposure, viz., 30 secs., and the rate of mortality was higher.

Evidence of the mechanical transference of DDT by flies is also presented.

Acknowledgement.

THE TYPE OF *PHLEBOTOMUS MASCITTII* GRASSI (DIPTERA, PSYCHODIDAE).

By Marshall HERTIG.

Gorgas Memorial Laboratory, Panama, R. de P.[1]

During the summer of 1948 Dr. Giuseppe Saccà, of the Istituto Superiore di Sanità, Rome, called my attention to the taxonomic vicissitudes undergone by *Phlebotomus mascittii* since it was described by Grassi in 1908. He showed me the pertinent literature which he had assembled, as well as specimens of *P. larroussei*, Lngn. & Nitzu., which both he (1940, 1948) and Parrot (1944) have maintained to be probably identical with *P. mascittii*.

Grassi described *P. mascittii* from several specimens, males and females, reared from larvae collected in a " cantina " (cellar) in the Via Panisperna, Rome. One male was sent to Newstead who in 1914 redescribed and figured it as *P. mascittii* together with a comparative series of drawings of the third antennal segment, wing and style of both *P. mascittii* and *P. perniciosus*, Newst. Newstead stated that these two species " are so closely allied as to be separable only with difficulty." While Grassi stated that the style bore five spines, Newstead's specimen had a sixth spine, finer than the others, on each style. Newstead pointed out that the wing venation is " strikingly different " in the two species and his figure shows clearly the venation which Grassi stated was constant in all his specimens. The length " dello scapo della forchetta sezionale," *i.e.*, *beta* of current usage, is approximately equal to the distance from the distal end of *beta* across cell R_1 to the end of vein R_1 (a distance greater than *delta* ; see Saccà (1940, 1948)). Newstead's small-scale drawing of the genitalia shows the tips of the aedeagus (penis sheath, intromittent organ) as rounded, quite unlike the forked tips of *P. perniciosus*. Newstead himself, in his original description of the latter species (1911) only three years before, had mentioned this character in the text and clearly figured it. It is strange that this point was not brought out by Newstead when he was obviously devoting considerable effort to the problem of distinguishing the two species. At any rate, he left them as two distinct species.

Adler and Theodor (1931a), in connection with their work on kala azar in Italy, found it essential to determine the systematic position of *P. mascittii* and *P. perniciosus*, and to that end undertook collections in the type locality of the former. Guided by Signor E. Mascitti, Grassi's former assistant, they visited the same house from the cellar of which the larvae of *P. mascittii* had been originally collected and caught 12 adults, all *P. perniciosus*. They stated that in their very large collections of sandflies in Italy there were many specimens, the males all five-spined, which corresponded to both Grassi's description of *P. mascittii* and Newstead's of *P. perniciosus*. They considered the sixth spine of Newstead's specimen as probably an abnormality which is known to occur rarely in other species. The matter of wing venation was not specifically discussed. They pointed out that the possibility that *P. perniciosus* was a synonym of *P. mascittii* could only be settled by a re-examination of the cotype.

Dr. Saccà and the writer made an evening visit in August 1948 to the Via Panisperna on the odd chance of picking up a specimen of this rare sandfly. Dr. Saccà had caught one of his specimens near this street (in the act of biting during the day) but no outdoor evening collecting had been attempted. This street, about 600 metres long, lies a short

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A

distance north of the Colosseum. For the most part it is solidly lined with apartment buildings several stories high. Signor Mascitti was no longer living and no one knew the exact location of the type house. However, we prowled about the grounds and outer walls of a large institution at the intersection with the Via Milano and caught two specimens of *P. perniciosus*. Through the friendly offices of caretakers several nearby apartment buildings were searched, both the upper floors and the very deep and extensive cellars, without finding any sandflies. Some of the residents' reports indicated that *Phlebotomus* was at times present and even annoying. We learned of no local application of DDT.

A fresh set of complications arose with the description of *P. larroussei* Lngn. & Nitzu. 1931 (syn. *P. vesuvianus* Adl. & Theo. (1931a), *P. perniciosus* var. *nitzulescui* Simić (1932) and its variety *canaaniticus* Adl. & Theo. (1931b). Saccà (1940), strongly suspecting the identity of *P. mascittii* and *P. larroussei*, called attention to the similarity of the wing venation and to the fact that the aedeagus in Newstead's drawing of *P. mascittii* corresponded to that of *P. larroussei*. Parrot (1944) believed that these two species were probably identical. Theodor (1948) still held possible the synonymy of *P. perniciosus* with *P. mascittii*, pending a re-examination of type material. On the basis of the wing venation of a number of specimens taken in Rome, and of Newstead's description, Saccà (1948) concluded that there could be no further doubt about the status of *P. larroussei* as a synonym of *P. mascittii*.

Grassi's specimens are not known to be in existence. Newstead's male would therefore represent the sole remaining specimen of the type material. In passing through London a visit was made on 27th October, 1948, to the British Museum (Natural History) for the express purpose of trying to find this specimen in Newstead's *Phlebotomus* collection, which it was known had been deposited there. Through the kindness of Mr. Paul Freeman, of the Department of Entomology, the entire collection, which filled a number of slide boxes, was placed at my disposal. The slide, which was one of the very few not indexed, was finally found (in the last box, among the Neotropical sandflies).

The slide bore Newstead's label, the seven lines of which read as follows : " *Phlebotomus* ♂/*mascitti* [sic] Grassi/Cotype ♂from Grassi/RNs. Paratype/ Remounted restained/RN Figd Bull. Ent./Research ". The last " RN " was Newstead's monogram with which he signed many of his drawings. On the opposite end of the slide were two labels, which were apparently Grassi's. One read : " Panisperna/28-7-08/ *Ph. Mascitti* [sic] " ; the other : " *Ph./Mascittii* ". On the coverglass was a small circular label, " Paratype ♂ ". With Mr. Freeman's permission a label was prepared and fixed to the back of the slide, stating that " Panisperna " referred to the street in Rome where Grassi had collected the larvae from which his specimens had been reared.

The specimen was compared directly with Newstead's drawings, which were substantially accurate. The tips of the aedeagus were smoothly rounded and bore no teeth or other projections of the sort which occur in some members of the *major* group. A camera lucida drawing (fig. 3) of the aedeagus was made. There was no possibility of confusion with the forked tips of the aedeagus of *P. perniciosus*. The abdomen had been detached from the rest of the body. The genitalia lay in the same position as drawn by Newstead (1914) (his fig. 3) from Grassi's original preparation before an accident which necessitated his remounting the whole specimen. In this process both of the small sixth spines had broken off, but their points of attachment were still clear. One of the large spines was also apparently lost and the position of two others disturbed.

Most of the available time had been consumed in finding the specimen and it was impossible to make further drawings or detailed notes, except for the puzzling circumstance that the genital pump was apparently lacking the cuplike expansion

at the anterior end, usually a conspicuous, well sclerotized structure in all species previously examined. There had been no mechanical damage to that part of the abdomen.

A letter was immediately dispatched to Dr. Saccà informing him of the results of the examination of this slide, for him to use as he saw fit.

The following day, whilst examining some sandfly literature at the London School of Hygiene and Tropical Medicine, Parrot's paper (1937b) with the drawings of the genital pumps of Old World species was encountered by chance. One of them had no cup or " pavillon ". On noting that this was *P. vesuvianus* Adl. & Theo. (=*P. larroussei*) there was immediately obvious the intriguing possibility that this character, interesting in itself, might serve as an additional link between *P. mascittii* and *P. larroussei*. Parrot noted in two papers (1937a, b) that his specimen (from Crete) was " dépourvue de pavillon," without further comment, although he pointed out in the latter paper in a general account of the anatomy of the genital pump that the " pavillon " serves as a muscle attachment.

A second visit was made to the British Museum. Careful adjustment of the light showed that the cuplike structure was present and of " normal " form, but thin-walled and colorless. Camera lucida drawings were made of the pump (fig. 2) and also of the entire genitalia (fig. 1). It may be remarked that in Newstead's fig. 3 the genital pump is shaped like a slender arrow-head and bears no particular resemblance to the specimen as at present mounted or to any " ordinary " genital pump. Pressed for time, it was impossible to make any drawings or detailed study of the head, nor unfortunately were any notes made. It is my recollection that the head was not mounted so as to show the cibarium or pharynx.

Adler & others (1938) stated that their single male specimen of *P. larroussei* from Crete had the " pavillon " as in other sandflies. In discussing the relation to this species of its variety *canaaniticus*, they made no mention of the pump. Parrot and Martin (1944) considered the varietal status of *P. larroussei* var. *canaaniticus* doubtful, since they could not separate their specimens of both sexes collected in Beyrut, from *P. larroussei*. They figured and described the " pavillon " as thick-walled, but made no mention of this structure in their comparison with *P. larroussei*. From Simić's figures (1932), the genital pump of *P. perniciosus* var. *nitzulescui* is of the usual type. The question thus arises as to whether faintness or " absence " of the " pavillon " is peculiar to the two specimens in which it has been observed or is, indeed, a character of specific or varietal value.

Leaving aside the question of the genital pump, and making due allowance for variations in the artists' drawing technique, there are no gross points of difference between the genitalia of Newstead's specimen and the various descriptions and figures of the male of *P. larroussei*. In all cases the tips of the aedeagus are smoothly rounded, with no teeth or other projections. In Newstead's specimen this organ is slightly more slender than in the others. Certain differences in proportions of the different parts of the male genitalia, as between their specimens and Parrot's, were discussed by Adler & others (1938). The proportions for Newstead's and Simić's specimens fall within that range of variation. Parrot's (1937b) ratio f/P (filaments/pump) is practically identical (about 3·7) for his, Newstead's and Simić's specimens, and not available for the others.

After returning to Panama the observations in regard to the genital pump of *P. mascittii* were sent to Dr. Saccà, together with tracings of the drawings and with the expressed intention of leaving any published comment to him, in view of his primary interest in the matter. However, the letter from London had reached him just in time for a postscript to his published note (1948). He requested that I publish these data, which would serve as additional support for his views.
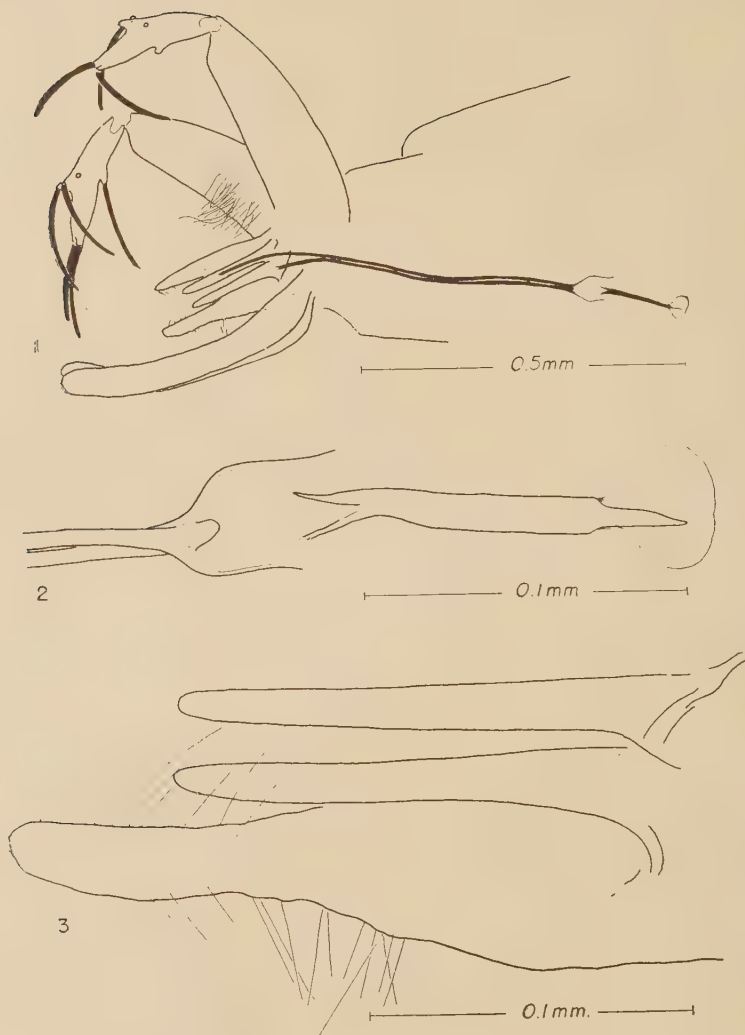
Phlebotomus mascittii ♂, drawn from Newstead's cotype, herein designated the lectotype, in the British Museum.

Fig. 1.—Genitalia. Corresponds closely with Newstead's (1914) figure 3. The small circles on the style mark the points of attachment of the probably aberrant sixth spines broken off when Newstead remounted the specimen. On the upper style one of the five large spines was apparently already missing; in remounting another was lost and two others bent sharply out of position.

Fig. 2.—Genital pump. The cuplike expansion at the anterior end is colorless and difficult to see, compared with other sandflies.

Fig. 3.—Aedeagus (penis sheath, intromittent organ) and paramere. The hairs of the latter are shown only in part, with no indication of their relative thickness or points of attachment.

Summary.

Examination of Newstead's specimen has confirmed the substantial accuracy of his description and drawings, with the exception of the genital pump. The form of the aedeagus alone leaves no possibility of confusion with *P. perniciosus,* of which there has been available an adequate series of Italian specimens for comparison. The view of Adler and associates that the sixth spines are aberrant is thoroughly reasonable. *P. mascittii* is therefore an easily recognizable species, as to the male, from existing descriptions and drawings, and represented by the sole known cotype in the British Museum, which is hereby designated the lectotype.

The question of the distinguishing characters of the female is bound up with the whole matter of the identity of *P. mascittii* and *P. larroussei.* The evidence already published, involving both males and females, certainly constitutes a strong case for the identity of these two species. There may be cited : (*a*) the character of the wing venation which seems to be constant in both sexes ; (*b*) the general agreement as to the male genitalia ; (*c*) the fact that one of Saccà's specimens was caught within a few hundred metres of the type locality and the others in the same city. In the absence of any contrary evidence there is no reason to doubt the soundness of the view shared by Saccà and Parrot that these two species are identical.

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STUDIES ON THE BIONOMICS OF THE SHEEP KED, *MELOPHAGUS OVINUS*, L., IN WEST WALES.

By G. Owen Evans, M.Sc., Ph.D.

Late of Section of Parasitology and Ecology, Department of Animal Health, University College of Wales, Aberystwyth.

The Life-cycle.

Studies on the life-cycle of the sheep ked, *Melophagus ovinus*, L., have been made in Australia by Sweet & Seddon (1917), Hill (1918) and Graham & Taylor (1941), in South Africa by Bedford (1926) and in North America by Imes (1917) and Swingle (1913). In Great Britain, published investigations on the ked are confined to a detailed study of the respiratory system (Webb, 1945) and to an account of a method of control (Heath, 1946).

The ked is a permanent ectoparasite of sheep and will not survive for more than two to five days when removed from the host. Observation of the life-cycle on live sheep has numerous disadvantages, in that the animals must be isolated singly and a careful search is necessary to find a small number of the parasites in the fleece. Hill (1918) succeeded in confining gravid females for a period of 24 hours in a metal ring (3 in. in diameter and 1 in. in depth) secured to the skin with pitch plaster : for longer periods this cage proved useless because the keds escaped after larvipositing. Other investigators were confronted with, and failed to overcome, the same difficulty.

A method, briefly described elsewhere (Evans, 1946), was devised to confine keds to an observable area of the fleece for a sufficiently long period to enable a number of investigations to be made on the life-cycle.

Technique.

A region of the fleece of the sheep in the form of a 4-in. square was isolated from the rest of the fleece by clipping a strip of wool to within $\frac{1}{8}$ to $\frac{1}{4}$ in. from the skin around the square. On this clipped strip a sleeve of mutton cloth (made by knitting cotton yarn with a plain stitch built upon itself) was secured by means of an adhesive mixture containing 80 per cent. rosin and 20 per cent. beeswax. The open end of the cage so formed was closed by means of a zip-fastener. In constructing the cage, care was taken to ensure that the area of the skin to which the cloth was secured was not disturbed by the limb movements of the sheep, because strains resulting from these movements would weaken the hold of the cloth to the skin and allow the keds to escape.

It was found necessary to reattach the cage after a period of seven weeks because a gap had by then formed between the skin and the cloth owing to the growth of the wool.

Four cages were attached to the flanks of two Welsh wethers, i.e. one cage on each flank of each wether. Daily recordings of the maximum and minimum temperature in the pens (unscreened) and the relative humidity of the sheep pen were made at 7.30 a.m. and 2.0 p.m. G.M.T.

Deposition of the first pupa.

The larva is deposited in an advanced state of development, and it pupates within six hours of deposition. In this paper, larviposition and pupation are regarded as coincidental.

The age at which a female ked deposits its first pupa was calculated by confining, in the same cage, a mature male with a newly emerged female. Although it was not possible to note the exact time at which copulation commenced, the pair were *in copula* sufficiently long (up to 24 hours) to be observed. Graham & Taylor (1941) stated that a young female is capable of mating within 24 hours of emerging from the puparium. During the present observations one young female was seen *in copula* 16 hours after emergence.

The time which elapses between copulation and the deposition of the first pupa is fairly constant. Twelve observations were made ; the minimum period was 12 days and the maximum 14, giving an average of 13·0 days. As can be seen in Table I, the duration of the gestation period of the first larva is not influenced by changes in the temperature and humidity of the air recorded during this experiment.

TABLE I.

The period after copulation at which the first pupa is deposited.

Month	Mean Temp. °F.		Mean relative humidity per cent.	No. of females observed	Period between copulation and deposition of the first pupa		
	Max.	Min.			12 days	13 days	14 days
Jan. ...	44·0	38·0	71·0	3	—	2	—
Feb. ...	47·0	39·0	72·5	3	2	1	—
Mar. ...	45·4	42·2	67·2	3	—	2	1
April ...	55·3	47·1	73·0	3	—	3	—

Deposition of the second and third pupae and the maturation period of the female.

The period elapsing between the deposition of the first pupa and the deposition of the second and successive pupae was observed by the following method. A female which had deposited its first pupa was immediately transferred into a cage with a mature male and the appearance of a pupa in the cage noted. From six observations it was concluded that the second pupa was deposited by the female 7 to 8 days after the deposition of the first, the average of six observations being 7·3 days. The gestation period of the third larva was observed by removing the females which had deposited their second pupae into a cage with mature males ; it proved to be 7 to 8 days, the average time for six observations being 7·5 days. These periods were not affected by any change which occurred in the temperature and humidity of the air during the observation period.

It appears from the above results that the gestation period of the larva of *M. ovinus* is about 7 to 8 days. The newly emerged female, which copulates 16 hours after emergence from the puparium, does not, however, larviposit until 12 to 14 days later. This implies that the young female is not sexually mature until the 6th to 7th day after emergence. This interesting observation was also made by Graham & Taylor (1941) and the fact that the female is able to copulate effectively soon after emergence appears to be due to certain peculiarities of the oviduct, described by Pratt (1899), which allow sperms to be stored until the ovum is ready to be fertilised.

Duration of the pupal stage.

The duration of the pupal stage was determined by two methods. Firstly, gravid females were collected and placed in a cage on the flank of a wether. The gravid female is distinguished by its enlarged abdomen, inside which the larva can be seen as a white mass when viewed ventrally. At the end of 24 hours the females had deposited their pupae and were removed from the cage. Daily observations of

the pupae were made in order to note the time taken for them to hatch. Secondly, newly deposited pupae located on the neck region of one of the wethers used in another experiment were differentiated by colouring the fleece around the pupae with different coloured dyes. The rate of development of individual pupae could then be ascertained.

From a total of 18 pupae deposited in the cage, 16 hatched. All 12 pupae hatched on the neck of the wether. In Table II it will be seen that the minimum period for development was 20 days, the maximum period 26, with an average of 22·5 days. According to Graham & Taylor (1941), there is an optimum temperature of about 86°F. for the development of the pupae, hence the fluctuation in temperature evident during the course of the present work probably accounts for the variation observed in the rate of development. Hill (1918) found the incubation period of the pupae in Australia to be 21 to 24 days in winter (Temp. 43° to 47°F.) while Graham & Taylor (1941) in the same country, recorded 20 to 22 days with a maximum of 30 days.

TABLE II.

Duration of the pupal stage.

Month	Mean Temp. °F.		Mean humidity per cent.	No. of pupae observed	No. of pupae hatching in the following times (days)						
	Max.	Min.			20	21	22	23	24	25	26
Jan. ...	44·0	38·0	71·0	5	—	—	2	2	—	1	—
Feb. ...	47·0	39·0	72·5	5	—	—	1	2	—	—	2
Mar. ...	45·4	42·2	67·2	6	—	1	2	2	1	—	—
April ...	55·3	47·1	73·0	7	—	1	4	2	—	—	—
May ...	57·4	49·0	68·3	5	1	3	—	1	—	—	—

Maturation period of the male.

Preliminary experiments by Graham & Taylor (1941) suggest that male keds reach sexual maturity 10 days after emergence. These investigators experienced great difficulty in observing the time at which mating took place because newly emerged males, placed with newly emerged females on the same sheep, were found to scatter quickly. The delay in mating may have been due to the small number of keds on the sheep with the consequent limited opportunities for males and females to meet.

In the present work several newly emerged male and female keds, collected from pupae hatching on experimental sheep, were confined in a cage on the flanks of the wethers. One cage was used to confine two pairs of keds. Daily examinations of the sheep showed that the keds did not attempt to copulate until the 10th or 11th day. In one instance a pair did not copulate until the 13th day. Pupae deposited as a result of the matings appeared 7 to 8 days after copulation.

TABLE III.

Maturation period of the male.

Date 1946	No. of male keds used	No. of keds *in copula* days after emergence				Rate of deposition of pupae in days, as a result of mating			
		10	11	12	13	17	18	19	20
13/3	4	2	2	—	—	1	2	1	—
28/3	3	1	1	—	1	1	1	—	1

The duration of the life-cycle.

During observations on the duration of the various stages in the life-cycle, the period taken for the complete life-cycle was obtained in five instances. The data obtained are shown in Table IV.

TABLE IV.

The duration of the life-cycle.

				Rate of development of the various stages				
Emergence of young female ked	...	...	14/1/46	22/1/46	7/2/46	4/3/46	28/3/46
Deposition of the first pupae	...	...	...	27/1/46	7/2/46	19/2/46	18/3/46	10/4/46
Emergence of the young ked	...	...	...	19/2/46	26/2/46	12/3/46	9/4/46	1/5/46
Duration of the life-cycle	...	...	...	36 days	35 days	33 days	36 days	34 days

The period elapsing between the emergence of the female and the hatching of the first pupa deposited is 33–36 days (an average of 34·8 days from five observations).

The significance of mating.

According to Graham & Taylor (1941), sufficient sperms are stored from one mating in the *receptaculum seminalis* of the female genital tract to last as a fertilising medium during its lifetime of approximately five months. These investigators succeeded in keeping fertilised females alive for 120 days on sheep free from male keds. During this period about 13 pupae were deposited by each female, while unmated females, kept for periods up to 25 days, did not deposit pupae.

In one of the present experiments four newly-emerged females were confined in a cage for a period of 30 days. During that time no pupae were deposited and so two mature male keds were introduced into the cage. One pair mated almost immediately while the second pair was found to be *in copula* when the sheep were examined 15 hours later. Four days after introduction into the cage the two male keds were removed, by which time all the females had mated. The first pupa was deposited in the cage on the 8th day after the introduction of the males, two more were observed on the 9th day, while the fourth was deposited on the 11th day. The cage was then re-attached and the same four females, without further mating, were confined for another 22 days. At the end of that period, 11 pupae were found in the cage, all of which hatched.

In another experiment, of a similar nature, four gravid females of different ages and four mature males were placed in a cage. All the females were found to have larviposited 16 hours later. When the cage was examined eight hours after larviposition, three pairs appeared to be mating. In two pairs copulation was in progress and, under a dissecting microscope, the posterior region of the abdomen of the male could be seen flexed underneath the abdomen of the female and the penis firmly implanted in the genital orifice of the female. In the third pair coition had not commenced, as the male left the female on being disturbed. Mating was not observed to take place in the fourth pair.

The above results support and extend the work of Graham & Taylor (1941). The female ked is not parthenogenetic and although from an initial mating a series of viable pupae can be deposited, further mating may occur.

The ability of the female to store sperms in its genital tract for use when there is no male present for mating has an important bearing on the rate of build-up of an infestation of a sheep. As transference is achieved almost entirely by contact, the presence of a single fertilised female is sufficient to produce a heavy infestation and this is of significance when elaborating control measures.

The effect of experimental conditions on the life-cycle.

By confining the movements of the ked to a limited area of the fleece, an unnatural environment may be created, possibly affecting the duration of the stages in its life-cycle. For the investigation of this problem, isolated ked-free sheep were used.

Four females, which had recently deposited their first pupa, and four mature males were placed on the shoulder region of a wether. Four pupae were found in the neck region of this wether, 7–8 days later. The average gestation period for these four female keds was 7·5 days which compared favourably with an average period of 7·3 days obtained from six observations by the cage method.

The rate of development of 12 pupae located on the neck region of a wether and referred to above (p. 461) was 21–25 days with an average of 22·2 days. The results again compared favourably with those obtained by the cage method on which the rate of pupal development was 20–26 days with an average of 22·5 days.

It therefore appears that the development of the pupae and the duration of gestation of the female was not affected by the experimental conditions to which they were subjected during the present work.

Incidence in a Flock of Welsh Sheep.

Our knowledge of the seasonal fluctuations in the infestation of sheep with keds is based on observations made by farmers, by many of whom it is believed that a heavy infestation of the ewes is built up during the late winter and spring months and that transference of keds to the lambs occurs during shearing. This transference is believed to take place as a result of the scant protection offered to the ked by the shorn fleece of the older sheep. The lambs, therefore, suffer from the ravages of the pest at an important period in their development, with consequent financial loss to the owner.

During the present investigation, the ked infestation of a flock of Welsh sheep was observed by making a periodic examination of individual sheep. The observations extended over a period of nine months from 21st January to 18th September, 1946.

Technique.

A flock of Welsh sheep, comprising 18 hoggs, six 2-year-old and six 3 year-old ewes, confined to a plot about ten acres in area, was examined approximately every two weeks. From 10th April, ten lambs, offspring of the older ewes, were examined also. The daily atmospheric temperature was recorded by means of a maximum and minimum thermometer placed about 2 ft. 6 ins. from the ground, near the centre of the plot. All the sheep were earmarked, so that a record of individual sheep could be kept during the period of observation.

Several methods of estimating the total number of keds on the sheep were attempted. The most satisfactory method involved the division of the fleece into six regions, namely the neck, forelegs, sides, hind limbs, belly and back, each of which was searched carefully for adult keds by parting the wool fibres with the fingers. The ked does not move very rapidly when disturbed, so that the danger of counting the same ked twice was negligible. Keds were found near the surface of the skin from January to April, but later in the season the majority occurred near the surface of the fleece. Before shearing, the examination of one sheep occupied about 30 mins. After shearing, when the fleece was short and the keds more easily seen, the examination of one animal could be completed in about ten minutes.

Fluctuations in the infestation of the flock.

In fig. 1 the infestation of the flock from January to September, 1946, is given.
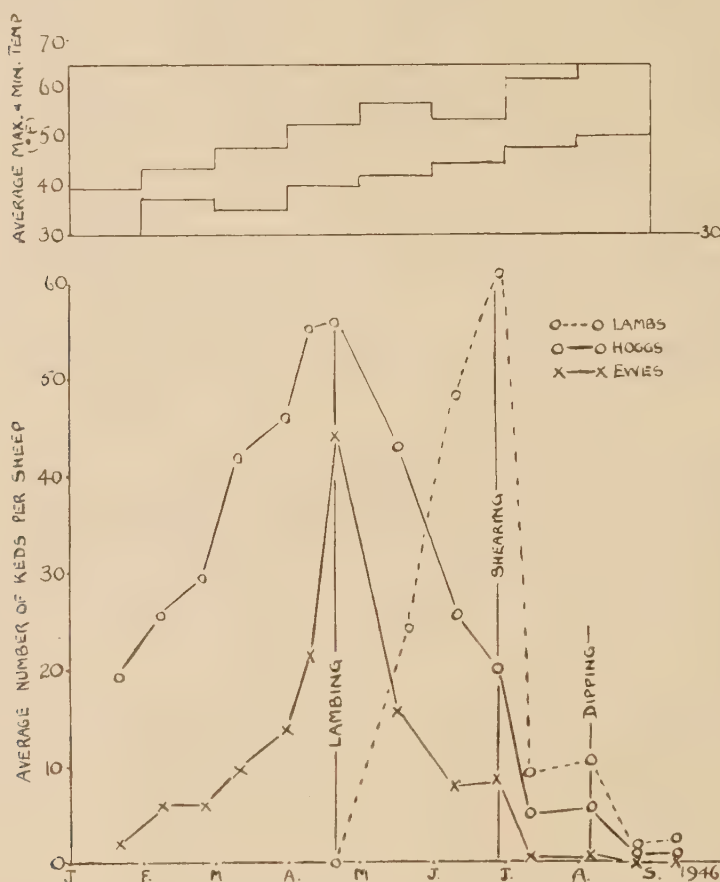
Fig. 1.—Fluctuations in the infestation between January and September, 1946.

At the first examination the hoggs were found to carry an average infestation of 19·6 keds, and the 2- and 3-year old ewes one of 2·3. Up to 7th April there was a steady increase in the infestation of the two groups and by this date the hoggs were carrying an average of 55·2 keds and the ewes one of 22·3. When the flock was examined on 15th April, there was a very slight increase only in the infestation of the hoggs, as opposed to an average increase of 22·3 keds (mean total infestation) in the ewes. This marked increase in the infestation of the pregnant ewes cannot be accounted for by the normal process of building up an infestation, by the emergence of keds from a reservoir of pupae in the fleece of the sheep (fig. 2). In the hoggs there should have been a substantial increase in the infestation owing to the emergence of keds from pupae deposited at the beginning of March, since the period for pupal development is 20–26 days (Evans, 1946). Between 7th and 15th April a transference of keds from the hoggs to the pregnant 2- and 3-year-old ewes must have occurred with a resulting heavy infestation of the ewes prior to lambing. This transference

was probably facilitated by direct contact between the sheep when penned for examination. The peak infestation of the pregnant ewes in mid-April of 44·5 keds had decreased to 16·0 by 15th May. Among the hoggs there was likewise a decrease to 42·8 keds. The ten lambs (born approximately a month earlier) examined on the same day carried an infestation of 24·7 keds. A rapid increase in the infestation of the lambs with a decrease in the infestation of the hoggs and the ewes continued until shearing on 20th June.
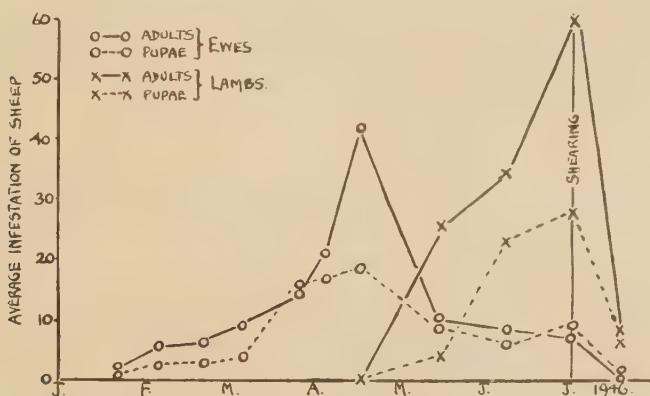
Fig. 2.—The infestation of ewes and lambs with pupal and adult stages between January and July, 1946.

The initial infestation of the lambs resulted from the transference of keds from the ewes. This transference must have continued for several weeks since, as is shown in fig. 2, the number of pupae present in the fleece of the lambs was insufficient to account for their continued heavy infestations. It was not possible to ascertain whether, during the period between lambing and shearing, keds were first transferred from the hoggs to the older ewes and then to the lambs, or whether transference was direct from hoggs and ewes. It is probable, however, that during the first month after lambing, when the lambs remained near their mothers, transference was from the latter. When the lambs began to wander among the other members of the flock, transference probably occurred direct from hoggs to lambs.

The whole flock was hand shorn on 20th June with the expected result of a rapid decrease in the infestation (Table V).

TABLE V.

The effect of hand shearing on the removal of pupal and adult keds.

Age group of sheep	Average number before shearing		Average number after shearing		Decrease per cent. as a result of shearing	
	Pupae	Adults	Pupae	Adults	Pupae	Adults
Lambs	31·0	60·3	9·4	9·0	69·7	85·1
Hoggs	17·7	20·9	2·2	5·2	87·6	75·1
2- and 3-year old ewes ...	10·2	9·0	0·6	0·8	94·1	91·1

The average decrease was 51·3 keds per lamb, 15·7 keds per hogg and 8·2 keds per 2- and 3-year old ewes. The actual number of pupae and adults removed is controlled by the type of shearing. If the fleece is shorn close to the skin, and particular attention is given to the neck and crutch regions, nearly all the pupae and adults are removed. In this particular instance, with hand shearing, only 69·7 per cent. of the pupae were removed from the lambs and this would result in a reservoir of pupae remaining to initiate a further infestation.

The whole flock was dipped in an arsenical preparation on 6th August. This resulted in a further decrease in the ked population on the sheep to an average of 1·8 keds per lamb, 0·3 per hogg and 0·1 per 2- and 3-year-old ewe. Following dipping, the infestation of the members of the flock began to increase normally until 18th September when observations were discontinued.

The transference of keds between sheep.

According to Graham & Taylor (1941) and Evans (1946), fluctuations in the atmospheric temperature had little or no effect on the life-cycle of the ked except in the rate of pupal development. It would be expected that the ked population of the sheep would continue to increase over the summer months unless certain measures, such as shearing and dipping increased the normal mortality rate by affecting the environment of the insect, and as long as the sheep were isolated to prevent transference from a heavily to a lightly infested animal. This steady increase does occur when sheep are isolated. In Table VI the infestation of three wethers isolated in a pen, is compared with the infestation of 18 hoggs of the same age and breed but running with lambs and older ewes. Unfortunately the three wethers had to be shorn early in August owing to their susceptibility to attack by the sheep maggot fly.

TABLE VI.

The infestation of isolated hoggs and of hoggs running with older ewes and lambs.

Type of sheep	Average ked infestation of the sheep from April to July, 1946			
	April	May	June	July
Isolated hoggs	68·2	97·4	123·3	187·6
Hoggs running with older ewes and lambs	55·5	43·1	23·8	5·2

The decrease in the infestation of the hoggs from April to July was the result of the transference of keds from the heavily infested hoggs to the lightly infested ewes. This transference was effected by direct contact and it was observed in the course of the present work that it is considerable when the keds are wandering over the surface of the fleece but negligible when they are confined to its depths. It is true also that keds often lose their hold of the fleece, drop to the ground and might be picked up by the other sheep. According to Hill (1918) the keds will not survive on the ground for more than four days and the chance of returning to their normal environment is slight. Unless sheep are closely confined, this type of transference is of little significance.

During the period 15th April to 5th July, it was noticed that a number of keds disappeared from the fleece of the animals, other than through transference to other sheep. For instance, of about 930 keds lost by the older sheep and hoggs about 640 were transferred to the lambs. These figures are only approximate as it was not possible to determine the normal mortality rate and the number of keds hatching

from pupae present in the fleece of these animals during this period. Thus, there was an approximate loss of 290 keds from 40 sheep during a period of 51 days. Graham & Taylor (1941) observed this disappearance of keds from their experimental sheep but were unable to account for it.

On several occasions, the writer has observed sheep biting keds from the surface of the fleece and then devouring them. This procedure was very marked during warm weather when some of them were on the surface of the fleece, and after shearing when the sheep were able to pick off keds without much difficulty. During colder weather, when the insects were in the depth of the fleece, the sheep had to be content with biting the fleece in an attempt to rid itself of the irritation. This observation is supported by Hoare (1923), who stated that about 80 per cent. of a flock of ked-infested sheep was infested with the protozoan parasite, *Trypanosoma melophagium* (Flu). Since sheep can only become infected with this parasite by ingesting the hind portion of the gut of an infected ked, the ked-eating habit of the sheep must be very common.

Two species of birds, the starling, *Sturnus v. vulgaris*, L., and the magpie, *Pica p. pica*, L., are often seen perched on the back of the sheep busily feeding on the insect fauna of the fleece. Whether or not these birds are an important factor in removing keds from the faeces of sheep presumably depends upon their numbers in the region where the sheep are grazing.

Influence of temperature and light on vertical migration in the fleece.

Since the transference of the ked between sheep generally occurs when the insect is on the surface of the fleece of its host, the following observations were made to assess the influence of temperature and light on the vertical migration of the ked in the fleece.
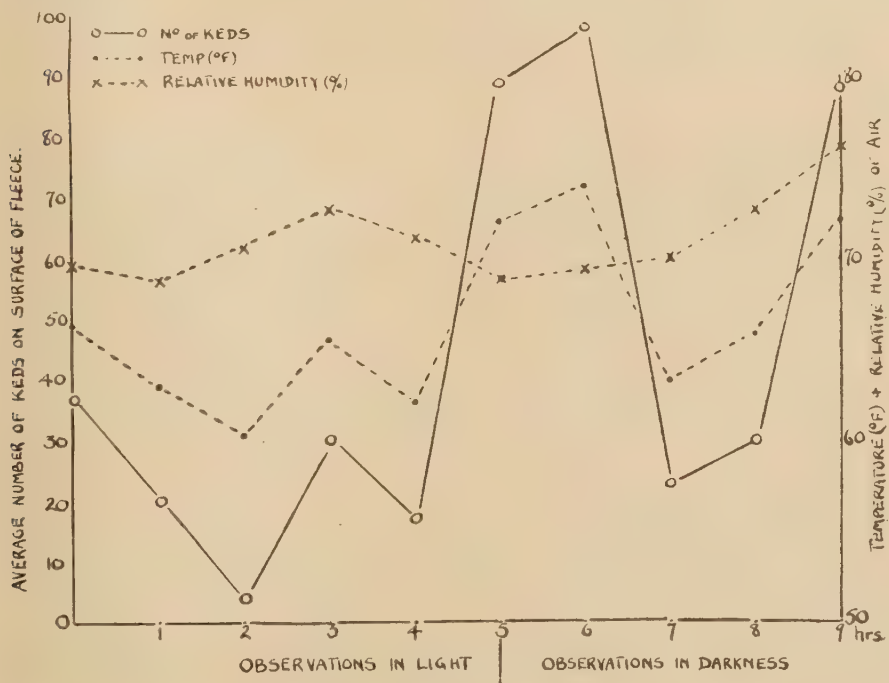
Fig. 3.—The effect of temperature and light on vertical migration in the fleece of hoggs.

Two heavily infested Welsh wethers were so penned that they could either be confined in a bricked shed or allowed to wander in a concrete yard. The temperature and humidity of the shed and yard were noted. The shed could be darkened or exposed to light, and changes in the temperature of the shed were brought about by means of a primus stove. Variations in the humidity of the shed could be controlled to a certain extent by allowing water to boil during the heating of the shed or by heating it in the absence of water. Thus by moving the sheep from the shed to the yard, they could be exposed to variations in temperature and light.

The effect of variations in temperature and the amount of light to which the sheep were exposed on the vertical migration of the ked in the fleece is shown in fig. 3. It is evident that, under the conditions of the experiment, the factor which controls this migration is the temperature of the air. The air temperature varied between 60° and 73°F. When the air temperature was 60°F. the number of keds on the surface of the fleece was small, an average of 4·0. When the air temperature was 73°F. there was an average of 98·0 keds on the surface of the fleece. At temperatures over 65°F., the migration was more rapid over a mean increase in temperature of 5°F. than for a corresponding increase below 65°F. These fluctuations in vertical movement were not affected by variations in the amount of light to which the sheep were exposed under the conditions of the experiment.

It is natural to expect, from the observations made above, that the temperature of the air is also an important factor in determining the period of transference. In fig. 1 the average maximum temperatures recorded between January and March were below 47°F. The highest temperature during this period was 62°F. on 31st March. This was an isolated high temperature, as other maximum temperatures were below 55°F. During these three months there was no evidence of transference of keds between the sheep. In April, however, the average maximum temperature was 52·2°F. and during the first 15 days of the month there were seven daily recordings of temperatures over 60°F. This increase in temperature coincided with the commencement of the transference of keds from the heavily infested hoggs to the lightly infested ewes.

The survival of the ked in a flock of sheep.

There are several factors which cause a high rate of mortality in the ked population of sheep during the course of the summer. The migration of the insect to the surface of the fleece renders it a prey to birds and to the sheep itself, while shearing and dipping result in a marked decrease in the infestation. The aim of the farmer is to eradicate the pest from his flock and although arsenical and various types of phenolic dips kill the adult population of the fleece, the pupae are resistant to treatment. Thus, a short period after dipping, young keds begin to emerge and it is these that form the nucleus from which the infestation of the sheep can be continued. Dips containing derris and DDT do, however, afford a complete eradication of the ked from the fleeces of sheep but unless the whole of the flock is dipped and segregated from other infested sheep, the flock will eventually become infested through transference of keds from the undipped sheep (E. P. Pollard, private communication, 1947).

Distribution on Welsh Sheep.

The distribution of the sheep ked over various regions of the host's fleece has not previously been examined in any detail. Several investigators, Swingle (1913), Hill (1918) and Graham & Taylor (1941), in the course of observations on the life-cycle, have noticed that certain areas of the fleece are more densely populated than others. Graham & Taylor (1941) support their observations on the distribution of the pupae of *M. ovinus* with quantitative data but they did not investigate the distribution of the adult at different seasons of the year.

Since the ked is a permanent ectoparasite of sheep, the condition of the fleece undoubtedly controls to a great extent the habits of the parasite. The nature of the fleece on different parts of the body varies considerably and it was in an attempt to note the effect of these variations, together with the difference in age and condition of the host on the distribution of the ked, that the following observations were made.

Technique.

In the course of fortnightly examination of 40 Welsh sheep comprising 10 lambs, 18 hoggs and 12 two- and three-year-old ewes, counts of the number of pupae and adult keds on specific areas of the body of these animals were made. For this purpose the body was divided into six areas as shown in fig. 4.
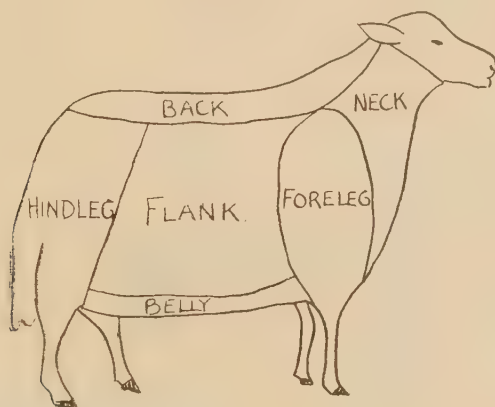
Fig. 4.

These regions were carefully searched for adults and pupae by parting the wool with the fingers, and to facilitate the counting of the pupae, which were numerous in certain regions, empty pupal cases were removed during each examination.

These observations continued from January to May in the case of the hoggs and the ewes. The lambs were examined during May and June.

Distribution in the fleece of hoggs.

The distribution of the adult and pupal stages of the ked on selected regions of the fleece from January to May is shown in fig. 5.

The variation in the relative numbers of the pupae in six regions of the fleece was very marked. Over 50 per cent. of the pupae were distributed on the neck region. The next most favourable position appeared to be that of the hindlegs, especially on the crutch, and then the forelegs and the flanks. Only 5 per cent. of the pupae were deposited in the belly region and over the six months, during which ten examinations were made, not one pupa was found on the back. The total number of pupae counted was 3,956. The findings of Graham & Taylor (1941) agree to the extent that the majority of the pupae are deposited on the neck region, but according to their observations, the next most favourable position is the " lower part of the sides, followed by the belly and the crutch ".

The variation in the density of the infestation of the adult on different regions of the fleece was not so clearly shown (fig. 5). Approximately 57 per cent. of the adults were found on the region of the forelegs and the flanks. The infestation then decreased through the neck, hindlegs and the abdomen. Only 12 per cent. of the adults were found in the latter region. The back, as in the case of pupae, gave a negative count. The total number of keds counted was 5,656.
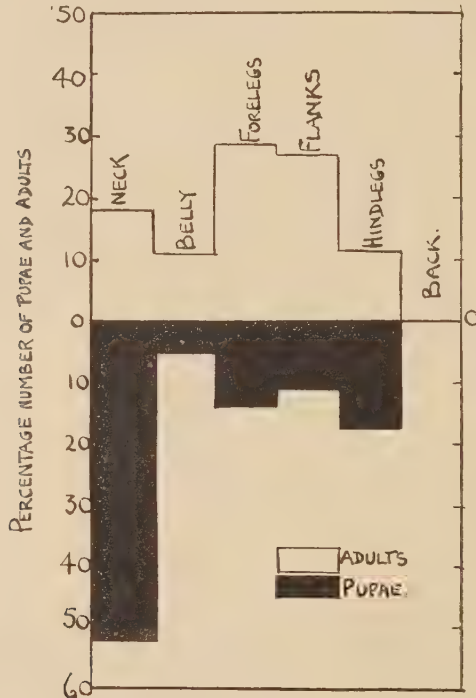
Fig. 5.—The infestation of various regions of the fleece of hoggs.

Periodic variations in the number of pupal and adult keds on hoggs and lambs.

In fig. 6 the infestation of 18 Welsh hoggs, from January to May is shown. The predominance of pupae and adults in the regions mentioned above is again clearly indicated. When the periodic fluctuations in the ked population of the six regions into which the fleece has been divided are examined, it is evident that a steady increase in all the regions occurred up to the middle of March. During April there was a marked decrease in the infestation of the neck, forelegs and flanks with a corresponding increase on the belly and hindlegs. There appears to have been a migration of keds to the belly and hindlegs, since the heavy infestation recorded in these two regions during April cannot have been built up from the reservoir of pupae deposited there.
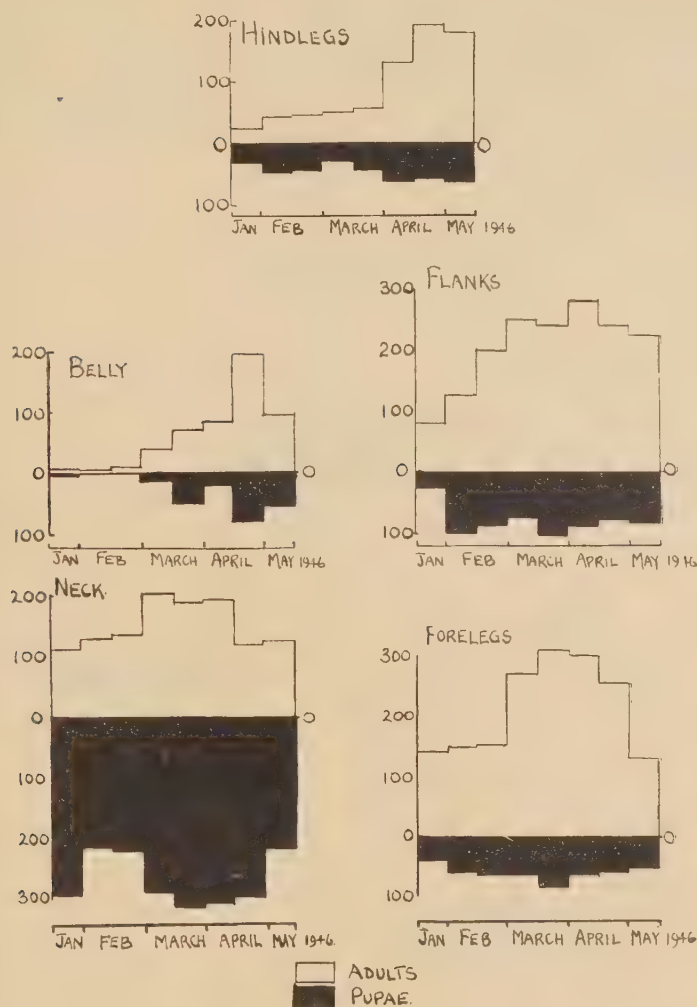
Fig. 6.—The periodic fluctuations in the distribution of pupae and adults on hoggs.

Pupal distribution is interesting in that the dense aggregation of pupae in the neck region does not result in the expected heavy population of adults. It appears that after emergence adults migrate from the neck to the forelegs and flanks but the majority of females return to larviposit in the neck region. At no time between January and May was the total number of pupae in the neck region of the eighteen hoggs below 300, whereas in the other regions the total number of pupae was always below 100. When the hoggs were examined on 15th May, the number of keds in all regions had decreased. This sudden decrease was due to the transference of keds from them to the pregnant two and three-year-old ewes· This phenomenon has previously been demonstrated on page 465.

The distribution of pupae and adults on the ten lambs is shown in fig. 7. These lambs were approximately three weeks old when first examined. Lambs build up a heavy ked infestation at an early age through contact with the parent ewe, but it is not until they are three or four weeks old that young keds emerge from pupae in their fleece. The most favourable position for the adult appears to be the hindlegs followed by the forelegs, belly, neck and flank. Pupae are concentrated on the fleece in the hindlegs, neck and belly although substantial numbers of adults are found on the flanks and forelegs.
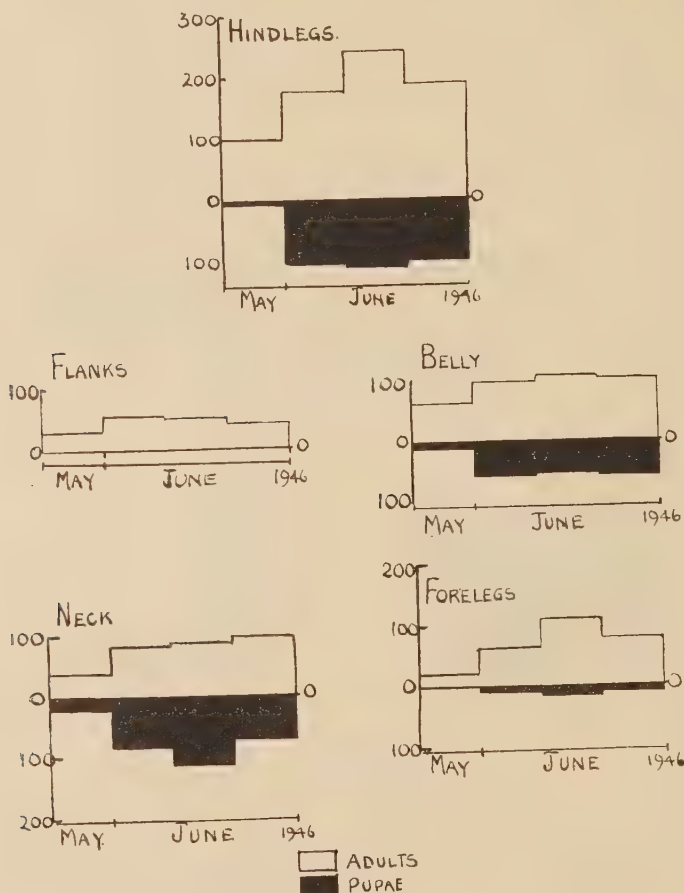
Fig. 7.—The distribution of pupal and adult stages in the fleece of lambs during May and June, 1946.

Location of pupae in the fleece of hoggs.

The fully grown newly voided larva, which becomes a pupa in about six hours, is completely motionless. The pupa is attached to the wool fibres at various distances from the skin that it was found extremely difficult to measure. The difficulty being due chiefly to the variation in the character of the staple over the whole fleece. In certain areas, especially the neck region, where the majority of

pupae were attached, the wool fibres which constituted the staple were greatly " crumped ", the degree of "crump" varying in different areas of the fleece.   The factor measurable with a relatively high degree of accuracy was the depth of the fleece.   This, together with the distance from the skin at which the pupae were placed, was measured with dividers, one point of the dividers resting on the skin and the other point level with the surface of the fleece or the pupa.   A total of 84 measurements were made during April on newly deposited pupae.

The relationship between the depth of wool and the distance of the pupa from the skin of the sheep is shown in fig. 8 and is expressed by the Regression coefficient $Y = 0.38 x - 45$.   The ratio of the distance of the pupae from the skin to the depth of the wool is seen to be relatively constant.   In the fleece, where the depth of wool is greatest, the distance from the skin at which the pupa is attached is greatest and *vice versa*.   The factor which controls the selection by the ked of a suitable point at which to deposit its larva seems to be the temperature in the fleece.   A series of experiments by Graham & Taylor (1941) on the viability of pupae at different temperatures and humidities appear to support this statement.   From their observations, they concluded that pupae will develop, *in vitro*, only under favourable temperatures and that the range is relatively narrow.   Variations in humidity do not appear to exert any marked effect at the temperatures tested by them.   In one experiment, Graham & Taylor incubated a large number of pupae over a wide range of temperature but at a constant humidity.   Development appeared to proceed normally only at temperatures between 78° and 93°F. the optimum temperature being about 86°F.
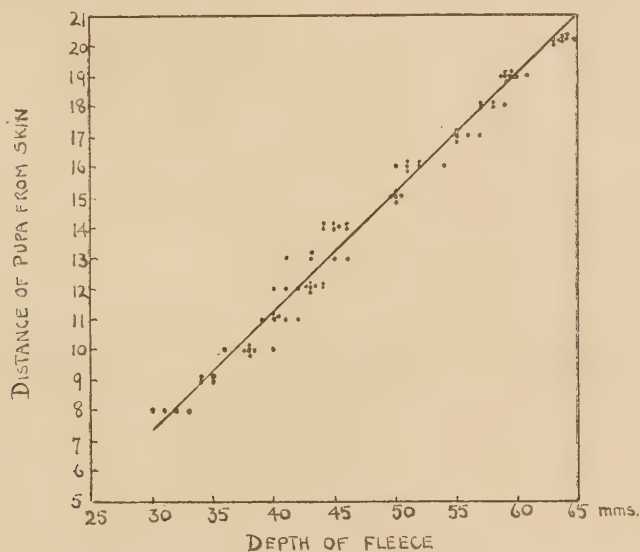
Fig. 8.—The region of attachment of the pupae in relation to the depth of the fleece.

During the present observations, the temperature of the skin of the sheep in the region of the neck was found to be about 99°F. while the temperature of the air near the surface of the fleece was about 59°F. giving a temperature gradient of 40°F. in a depth of 20 mm. of fleece.   In order that the pupae should develop normally, the larva must be deposited in the fleece where the temperature is favourable.   Owing to variations in atmospheric temperature, the temperature where larviposition occurs will not remain constant throughout the period of pupal development.   There is,

however, a sufficiently wide range of temperature in which the pupae will develop to cover this variation. The extent to which the pupa is exposed to temperatures varying from the optimum will control the rate of pupal development and account for the wide range in the duration of the pupal stage observed during investigations into the life-cycle of the insect.

Relation of infestation to type of fleece.

Among a flock of sheep of the same breed there is a marked individual difference in the length, texture and lanolin content of the fleece. Imes (1917) during observations on the life-cycle, noticed that breeds with open fleeces showed a higher degree of infestation than fine wool breeds. A quantitative study of the degree of infestation of Welsh hoggs of different fleece types has been made by the present investigator with the assistance of three expert judges of Welsh sheep who graded the fleeces into the following three main types :—

(a) Fine, greasy, short fleece

(b) Open, slightly greasy, long fleece

(c) Intermediate between types (a) and (b).

Only those sheep in which there was complete agreement as to the type of fleece were included in the experiment.
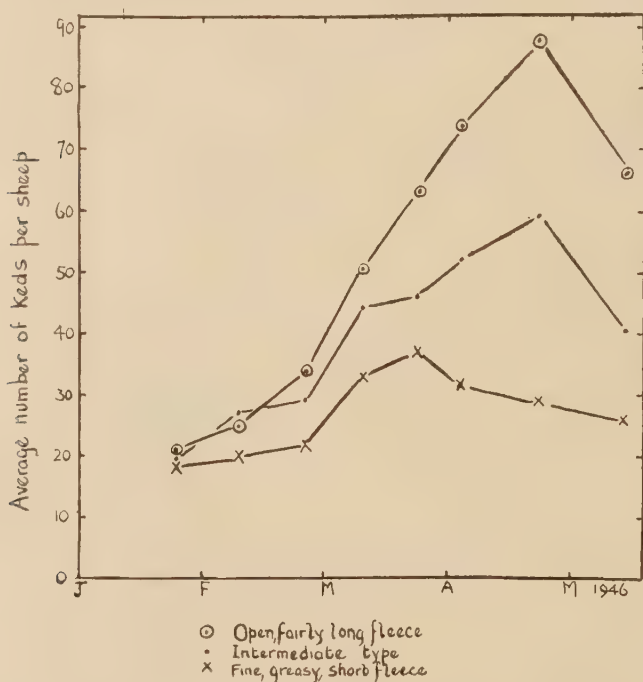
Fig. 9.—The effect of the type of fleece on the degree of infestation.

The average ked population on the hoggs in the three groups is shown in fig. 9, and it will be seen that there is a marked variation in the infestation of the hoggs of the three fleece types. The most favourable fleece type, from the aspect of

infestation, is the open, fairly greasy, long fleece. The fine, greasy, short fleece does not appear to provide such a favourable environment. In the fleece type (*a*) the movement of the ked would probably be easier and more rapid than in the case of fleece type (*b*) where there is no definite gap between the staples as in the open fleece. In the greasy fleece, also, there is a danger of the spiracles of the insect becoming covered with lanolin and thus providing a less suitable environment than the less greasy fleece. According to Milne (1945) the grease content of the fleece "increases from March to May as the pasture improves and becomes less viscous in the warmer weather". This may account for the relatively low infestation of the greasy fleeced hoggs from February to May as compared with that of the slightly greasy fleeced hoggs over the same period and from a similar initial infestation (in January the average infestation of type (*a*) was 19·7 keds and group (*b*) an average of 19·1 keds).

The variation in the depth of the fleece in the types mentioned is important when considering the relative suitability of the fleece for pupal development. In the short woolled type the distance along the wool fibres where pupal development can proceed normally is more restricted than in the long fleece type, and the danger of the pupae being exposed to adverse temperatures, resulting in their failing to develop, is correspondingly increased.

The variation in certain characters of the fleece, outlined above, is also evident in the fleece of the individual. On the neck region of Welsh sheep, the fleece is generally long and open, there is then a gradation in the length and looseness from the neck through the forelegs to the flank and hindlegs to the fairly short, fine wool of the back and the short coarse wool of the belly. Thus, for reasons given above, larviposition should occur in the regions where the fleece is long and adults would prefer the more open regions. This distribution does occur as is shown in fig. 5, where over 50 per cent. of the pupae are located on the neck region, about 45 per cent. on the forelegs, flanks and hindlegs, 5 per cent. on the belly region and none on the back. The distribution of the adult follows a similar pattern although it is more difficult to interpret owing to the migration of the insect in the fleece.

Age of sheep and infestation.

It has been previously noted that there is a variation in the infestation of the different age groups of sheep comprising a flock. This variation is most apparent when comparing the infestation of hoggs with that of older ewes (fig. 10). It is probable that the heavier infestation of the hoggs recorded by the writer was due to the prevailing husbandry methods. Lambs at an early age build up a heavy ked infestation at the expense of other members of the flock and after hand shearing the fleece of the lambs harbours more pupae than those of the older ewes. When the flock was dipped in an arsenical preparation, the pupae and many of the adults survived the treatment. This resulted in a heavier infestation of the lambs which would continue throughout the winter. Thus when the flock was examined in January the "lambs" (now classified as hoggs) would have a higher ked infestation than older ewes. The heavier infestation of the lambs after dipping should not occur when the use of DDT (αα dichloro-diphenyl βββ trichlorethane) and benzene hexachloride has become more widespread : both these insecticides afford a complete eradication of the ked if the treated sheep are segregated from ked infested sheep.

The sudden increase in the infestation of the ewes during April is the result of transference of keds from the hoggs to the ewes.

○ Hoggs.
X 2 yr. old ewes.
• 3 yr old ewes.
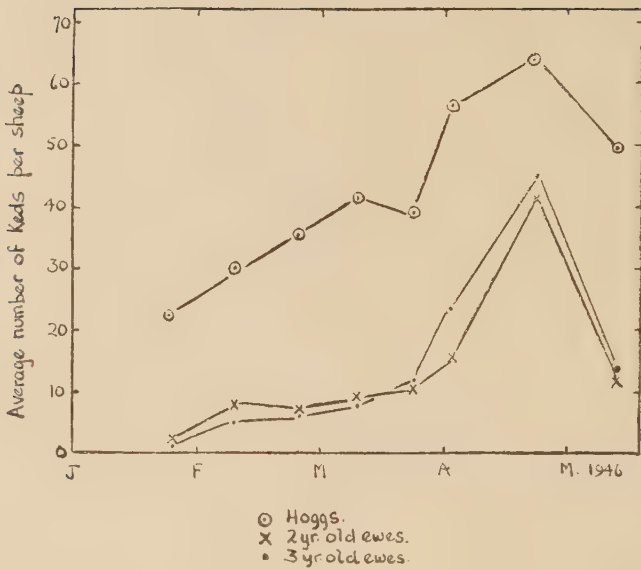
Fig. 10.—The infestation of three age groups of sheep between January and May, 1946.

The adaptability of the ked.

From the present observations, it is evident that the sheep ked has become highly adapted to the specialised environment afforded by the fleece of its host. It not only shows a marked adaptation in its larviparous habit, but it has succeeded in using the variations in the fleece type, evident over the body of the sheep, to overcome some of the difficulties caused by adverse weather conditions. The duration of stages in the life-cycle of the insect in various countries is shown in Table VII.

TABLE VII.

The duration of stages in the life-cycle reported from various countries.

Region	DURATION IN DAYS OF STAGES OF THE LIFE-CYCLE					Authority
	Maturation period		Deposition of pupae		Pupal stage	
	♂	♀	First	Succeed-ing		
Australia	—	—	13–23	7–8	19–24	Hill (1918)
	10	6	14–15	6–8	18–30	Graham & Taylor (1941)
S. Africa ...	—	—	13–14	10–12	19–26	Bedford (1926)
N. America ...	—	—	14–30	7–8	19–36	Swingle (1913)
Britain ...	10–11	6–7	12–14	7–8	20–26	Evans (1946)

The consistence of the duration of stages in the life-cycle is evident. The wide variation in the period taken for the newly emerged female to deposit its first pupa, as observed by Swingle (1913) and Hill (1918) is undoubtedly due to their mating males of various ages with newly emerged females. Graham & Taylor (1941) and Evans (1946) found that the maturation period of the male ked was 10–11 days as compared to 6–7 days for the female. Thus, when a newly emerged female is mated with a mature male, the female deposits its first pupa in about 13 days, whereas when a newly emerged male is mated with a newly emerged female the first pupa is not deposited until about the twentieth day.

The limiting factor in the geographical distribution of the ked is undoubtedly air temperature. Graham & Taylor (1941) report a decline in the infestation in Australia during the summer months, although the sheep had not been shorn or dipped. This increased mortality rate during the summer months was not observed by the writer when the maximum temperature recorded in July was 69°F. as compared with the 100°F. recorded by Graham & Taylor. The vertical migration of the ked in the fleece of its host observed by the writer at temperatures over 60°F. is not evident in Australia where Graham & Taylor were unable to correlate with the weather conditions, the movement from sheep to sheep, which occurs when keds migrate to the surface of the fleece. Swingle (1913) in Wyoming, however, observed on hot summer days fifty ticks (keds) crawling near the surface of the wool, while on cold days not one was in evidence. Temperature probably explains why the ked has not become established on sheep kept permanently in the moist tropical areas of the world, where according to Bequaert (1942) they die very soon after importation. The same authority states that at higher altitudes in the tropics, where the temperature is lower, such as in Kenya and on the slopes of the Andes, the ked is a permanent ectoparasite.

Summary.

A method is described for studying the life-cycle of *Melophagus ovinus*. The female ked matures in 6 to 7 days and the male in 10 to 11 days. Copulation takes place 16 hours after emergence and the first pupa is deposited by the female about 13 days after its emergence. The second and successive pupae are deposited at intervals of seven to eight days. The pupal stage covers 20 to 26 days (an average of 22·5 days for 28 observations). The life-cycle is completed in 33–36 days. Experimental conditions did not affect the duration of the various stages observed in the life-cycle.

An investigation of the periodic fluctuations in the infestation of a flock of Welsh sheep showed that the degree of infestation is influenced by the transference of keds between the sheep. Before lambing, keds are transferred from hoggs to two- to three-year old ewes and, after lambing, from ewes to lambs. The peak infestation of ewes and hoggs occurs before lambing. The maximum infestation of lambs occurs before shearing. At shearing the majority of adult keds and pupae are removed with the fleece. Adults surviving after shearing are killed at dipping. Depending on the nature of the dip, re-infestation results from pupae hatching in fleece or by the transference of keds from infested sheep coming into contact with the dipped flock. Keds disappear from the fleece through being devoured by the sheep, by the activity of insectivorous birds and through natural death. The transference of keds between sheep is achieved through contact and occurs readily when the ked is on the surface of the fleece. The vertical migration of the insect in the fleece is controlled by temperature and is not influenced by light.

The distribution of pupal and adult stages of *M. ovinus* in the fleece of lambs, hoggs and ewes is given. The position of attachment to the pupae is controlled by the depth of the fleece, deposition occurring on the wool fibres at a point where the temperature is suitable for their development. The age of the sheep influences the distribution of the ked, young sheep being more susceptible than older animals. In

a flock, the higher infestation of hoggs compared to older ewes may be due to the higher initial infestation of the hoggs after dipping. Open fleeced sheep are more susceptible to ked infestation than tight fleeced sheep.

Acknowledgements.

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THE INTRODUCTION OF *PHYSONOTA ALUTACEA* BOHEMAN (COL., CASSID.) INTO MAURITIUS.

By J. R. Williams, B.Sc., D.I.C., F.R.E.S.

Department of Agriculture, Mauritius.

A campaign to control the weed, *Cordia macrostachya* (Jacq.) Roem. & Schult. (Boraginaceae) by biological means is in progress in Mauritius (Williams, 1948). This hardy perennial shrub was accidentally introduced from Central America in the last decade of the nineteenth century. It is today a serious pest of nearly all land of agricultural value which is not intensively cultivated (Wiehe, 1946).

The first insect species received in Mauritius as a possible controlling agent was *Physonota alutacea*, Boh., which is indigenous to the Central American region. Food specificity tests both in Trinidad (Simmonds, 1949) and in Mauritius (Moutia, 1947) showed that this species could be released without danger to other plant species, and between 11th June 1947 and 28th May 1948, a total of 21,000 adults, 4,000 larvae, and 280 egg batches (approximately 7,000 eggs) were released in various localities over the island (Williams, 1948).

It very soon became evident that *Physonota* could not develop normally in the field. The released adults were often found copulating and ovipositing several weeks after liberation, but larvae were only found on a very few occasions. These were first-instar larvae which had obviously recently emerged, and they would invariably disappear within 24 hours.

The reason for the consistent disappearance of the young larvae was investigated. In Trinidad, the distribution of *Physonota* is extremely restricted, and it is suggested by Simmonds) *loc. cit.*) that the predacious Red Ant, *Solenopsis geminata*, F., is responsible. *Solenopsis* is widespread in Mauritius and observations have shown that it must account for a number of *Physonota* larvae. The smaller larvae are also carried off by the ant, *Pheidole megacephala*, F.

In order to determine whether *Physonota* could complete its life-cycle in the open when predators, and possible predators such as wasps of the genus *Polistes* and birds, are excluded, a *Cordia* bush was completely enclosed with mosquito netting. As there was a nest of *Solenopsis* in the vicinity, a circle of soil around the bush and its netting was treated with benzene hexachloride.

In spite of these precautions, first-instar larvae placed on the bush would disappear overnight. Some were taken by Salticid spiders, but the number so absconded was not considered to be large. The only insects on the bush were Black Ants, *Technomyrmex detorquens*, Wlk., against which benzene hexachloride is ineffective, a non-predatory species which attends *Cordia* bushes for its nectar and for the juices exuded by the urticating hairs.

It was concluded that *Technomyrmex* must play some rôle in the disappearance of the larvae, and a grease band was applied to exclude them. Under these conditions, first-instar larvae placed on the bush would develop to the last larval instar.

In further similar experiments, some leaves grew into contact with the netting, thus allowing *Technomyrmex* access to the bush. As a result, *Physonota* larvae again disappeared from the foliage, while examination of the grease band showed numbers stuck at the *lower* edge. It is concluded that *Technomyrmex* disturbs the small larvae so that they fall to the ground where they perish and that those that regain the foliage are once more ejected.

Technomyrmex is commonly found fostering Coccids, and is a limiting factor in the development of their Coccinellid predators. Its rôle in this respect has been confirmed by the control obtained over *Icerya seychellarum*, Westw., by the application of a grease band to infested plants (Moutia, 1935). This activity of *Technomyrmex* is comparable with its action on *Physonota* except that in the latter case it inhibits predators of a food plant instead of those of a food insect.

Cordia bushes in 18 localities over the island were examined for ants. The following species were recorded :—*Technomyrmex detorquens*, Wlk., *Pheidole megacephala*, F., *Tetramorium simillimum*, F. Smith, *Monomorium floricola*, Jerd., *Paratrechina longicornis*, Latr., *Anoplolepis longipes*, Jerd., and *Camponotus grandidieri*, Forel. Of these, *Technomyrmex* was found in all but five localities, and does not seem to be environmentally restricted in Mauritius. It is usually extremely abundant when present and in such cases it is to be noted that other species are either absent or are only occasionally seen on the bushes, thus affording further evidence of the dominating influence of this species. When *Technomyrmex* is absent, its place is taken either by *Pheidole megacephala*, which in addition to its predatory habit also feeds upon plant juices, or by *Tetramorium simillimum*. Otherwise the abundance of these ants on the bushes seems to be in an inverse proportion to the abundance of *Technomyrmex*. *Camponotus grandidieri* is thought to be predacious, while the remaining species listed are not commonly encountered on *Cordia*. It is not known if these species affect *Physonota*.

In addition to the effect of *Technomyrmex*, *Solenopsis*, *Pheidole*, and Salticid spiders on *Physonota*, egg batches were sometimes found to be damaged by an unknown predator. It is, however, the considered opinion of the writer that *Technomyrmex* is the primary cause of the failure of *Physonota* in Mauritius.

It is worthy of note that *Technomyrmex* was not known in Mauritius until about 1925, while *Solenopsis* was introduced about 1900 (d'Emmerez de Charmoy, 1922).*

My thanks are due to Mr. R. Mamet for his assistance in the identification of the various ant species.

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*Since the above was written, a very small colony of *Physonota* has been found at one liberation site, where adults and larvae were confined to within about 15 yards of the point of release. In this area ants, with the exception of *Camponotus*, were absent. This interesting discovery confirms the conclusions reached above.

A NEW SYSTEMIC INSECTICIDE
BIS(BIS DIMETHYLAMINO PHOSPHONOUS) ANHYDRIDE.

By W. E. Ripper, Ph.D., R. M. Greenslade, Ph.D., and G. S. Hartley, D.Sc.

(Plates VIII & IX.)

Schrader (1947a), working on the preparation and insecticidal evaluation of certain phosphorus and fluorine compounds, developed some that were shown to be systemic. A systemic compound is one that is absorbed by the plant and translocated in the sap so that parts of the plant other than those treated become toxic to sucking insects. Schrader referred to these substances as agents for the chemotherapy of living plants. One of them was bis(bis dimethylamino phosphonous)anhydride, which was prepared by him in 1942 and shown by Kükenthal (in Schrader, 1947a) to possess systemic properties. Dr. H. Martin (1947) called attention to these interesting compounds, pointing out (1949) that in regard to toxic hazards, bis (bis dimethylamino phosphonous)anhydride seemed more promising as an insecticide than some of the fluorine compounds.

At Dr. Martin's suggestion, a comprehensive investigation was undertaken to examine the entomological, physico-chemical, chemical, plant physiological and toxicological properties of this anhydride in order to assess the potentialities of systemic insecticides as represented by it. This investigation included a study of the toxic risks involved and the necessary measures to overcome them. The project was begun in 1947 and was carried out by the following workers :—

Biological Research : L. A. Lickerish, M.A., B.Sc. ; E. G. Goscombe, B.Sc. ; J. W. Cowland, B.A. (Agric.) ; W. Heatherington, B.Sc. (Agric.) ; D. G. Ashby, M.A., F.R.E.S. ; G. H. Bunzli, D. Tech. Sc. (Ent.) ; D. W. Mollison, B.Sc. and J. P. Tunstall, Dip. Ag.

Chemical Research : C. H. Barker, M.A. ; D. W. Pound, B.A., and D. F. Heath, D.Phil., B.A., B.Sc.

Medical Research : C. Webb, L.M.S.S.A., and H. Siedek, M.D.

A detailed description of the experimental work will be published elsewhere by some of the above ; the present report is a compilation of the main results and these are published so that they may become available with as little delay as possible.

Chemical and Physical Properties.

Bis(bis dimethylamino phosphonous)anhydride has the empirical formula $C_8H_{24}O_3N_4P_2$ (molecular weight, 286) and the structural formula

$$(CH_3)_2N \quad O \qquad O \quad N(CH_3)_2$$
$$P - O - P$$
$$(CH_3)_2N \qquad \qquad N(CH_3)_2$$
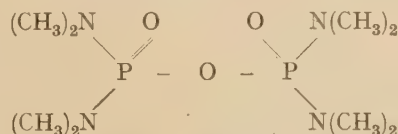
and may be regarded as pyrophosphoric acid in which the four OH groups are replaced by dimethylamino groups. Alternative names for it are tetrakis dimethylamino phosphonous anhydride and octa-methylpyrophosphoramide. It was first prepared by G. Schrader in the I.G. Laboratories in Germany.

The compound is normally a dark brown liquid with a viscosity rather less than that of olive oil and a slight odour. A highly purified specimen was of pale straw colour and solidified on cooling, its melting point being 14–20°C. The specific gravity of the pure compound is 1·09 25°/4°C.

The compound is miscible in all proportions with water and most organic solvents. It is only slightly soluble in refined mineral oils and incompletely miscible with vegetable and crude mineral oils. It partitions very heavily in favour of water, between water and mineral, aromatic or vegetable oils, but it is extractable from water by chloroform, partitioning about 7 : 1 in favour of the latter from a dilute solution in pure water, and more strongly so from aqueous caustic soda. Its extractability by chloroform is utilised in analysis.

Strong caustic soda causes partial separation of the compound from water at 100°C. Most other electrolytes do not cause separation but have their own solubility reduced. Amine phosphate, which is of interest as the hydrolysis product, is an exception to this.

The dry compound is for all practical purposes indefinitely stable ; it is slowly hydrolysed in dilute solution in water. Alkali slightly increases the rate of hydrolysis and hydrolysis is catalysed in a marked manner by acid. The first stage in the acid hydrolysis is hydrolysis of one N-P linkage, rapidly followed by several reactions leading finally to complete hydrolysis to dimethylamine and orthophosphoric acid. Pyrophosphoric acid does not appear. Under constant acid conditions, the disappearance of the active compound follows the unimolecular equation, the constant being $2\cdot3\times10^{-2}\times(H^+)$ min^{-1} at 41·3°C. and $4\cdot8\times10^{-3}\times(H^+)$ min^{-1} at 25°C. The half life at pH 1 is thus 5 hours at 41°C. and 14 hours at 25°C. At pH 2 it is 2 days at 41°C. and 6 days at 25°C. At pH 4 and above, the rate is determined by the " water reaction " rather than the acid reaction. This " water reaction " is thus the significant one for storage stability. It has a higher temperature coefficient and the limits for 10 per cent. decomposition are more than 5 weeks at 40°C. and more than 6 months at 20°C. If the compound is only slightly wet, hydrolysis of part of it to amine phosphate causes separation into two layers at high temperatures, the salt removing much of the remaining water as a separate layer and thus slowing down the subsequent reaction.

The volatility is low. Extrapolating from the boiling points obtained in vacuum distillation, the vapour pressure is estimated to be about 0·001 mm. of mercury at 20°C. Owing to the very high solubility in water, the vapour pressure of the compound arising from green plants will be much less, being that of a very dilute solution in water.

The compound has only a slight taste ; a solution of 1 per cent. is faintly reminiscent of black pepper, but a solution of 0·1 per cent. does not taste at all.

Formulation.

Three formulations of bis(bis dimethylamino phosphonous)anhydride have been tried out in the field :—

1. An aqueous solution containing 30 per cent. of the anhydride and no wetting agent. This solution should not be stored longer than one year.

2. An anhydrous formulation containing 66 per cent. of the anhydride and sufficient wetting agent to wet hop leaves ; it keeps indefinitely. (This formulation is frequently referred to hereafter, as formulation 2.)

3. An anhydrous formulation which contains 75–80 per cent. of the anhydride and keeps indefinitely.

For spraying purposes, 6 ozs. of Lissapol N (a wetting agent containing poly-ethylene glycol) per 100 gallons of diluted solution, or some other similar wetting agent, was added to the first formulation. In the case of the first two formulations methyl violet was also included as a warning colour.

Insecticidal Effect.

Bis(bis dimethylamino phosphonous)anhydride has a very weak direct contact effect against Aphids, and against other insects the contact effect is negligible. There is no contact action by a residual film, and no fumigation effect, either of the chemical itself or from sprayed plants. The systemic action is most marked against Aphids but other sucking insects and spider mites can also be destroyed. The following species have been controlled :

Species	Host plant	*Concentration of bis(bis dimethylamino phosphonous) anhydride
		Per cent.
Macrosiphum euphorbiae, Thomas	Sugar beet	0·5
Macrosiphum rosae, L.	Rose	0·1
Macrosiphoniella sanborni, Gill. (see fig. 1) ...	Chrysanthemum ...	0·1
Acyrthosiphon onobrychis, Boy.	Peas	0·1
Phorodon humuli, Schr.	Hops	0·1
Capitophorus fragariae, Theo.	Strawberry ...	0·1
Myzus persicae, Sulz.	Beet	0·5
	Tobacco	0·5
Aulacorthum circumflexum, Buckt.	Beet, Cineraria ...	0·5
	Chrysanthemum ...	0·1
	Hydrangea ...	1·0
Brevicoryne brassicae, L.	Cabbage	0·25–0·5
Aphis fabae, Scop.	Beet	0·5
Aphis pomi, Deg.	Apple	0·3
Aphis laburni, Kalt.	Groundnuts ...	0·05
Yezabura malifoliae, Fitch	Apple	0·3
Brachycaudus cardui, L.	Cineraria	0·5
Eriosoma lanigerum, Hsm.	Apple	0·5
Aleyrodes proletella, L.	Cabbage	1·0
Pseudococcus citri, Risso	Coleus, Chrysan-themum	0·1
Typhlocyba spp.	Potato, Apple ...	0·1
Empoasca lybica, Berg.	Cotton	0·3
Tetranychus telarius, L.	Hops	0·1
Paratetranychus pilosus, C. & F.	Apple	0·1

Systemic Effect.

The systemic action has been demonstrated by numerous experiments. An insecticidal effect on the upper parts of potted plants resulted from the " watering " of the roots with a solution of the compound ; Aphids on cineraria, potato, cabbage, brussels sprouts, beet and chrysanthemum were killed when the roots were " watered " in this way. Application to the upper surface of leaves killed Aphids feeding on the lower surface ; species thus killed were *Aphis fabae*, Scop., *Myzus persicae*, Sulz., on sugar-beet seed and on mangels ; *Brevicoryne brassicae*, L., on brussels sprouts ; *Macrosiphoniella sanborni*, Gill., on chrysanthemum ; and *Pseudococcus citri*, Risso, on coleus. Painting one-half of the leaf of brussels sprouts produced an insecticidal effect against the cabbage aphis on the other side of the mid-rib.

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*The concentration of bis(bis dimethylamino phosphonous)anhydride is calculated on the basis of 66 per cent. anhydride. In the case of *M. sanborni* 60·5 per cent. anhydride was used.

Spraying the lower leaves only of flowering cabbages, potatoes and chrysanthemum cuttings destroyed Aphids on the flower stems, the " tips " and the remaining leaves, respectively. The insecticide was translocated about three feet in the cabbages and one foot in the potatoes. Treatment of all the leaves of cinerarias resulted in the death of Aphids on the flowers.

Whilst adequate absorption through the root requires application to the soil of a considerable quantity of the systemic insecticide, absorption by the leaves and other aerial parts is much more economical and can be used for Aphid control on a large scale.
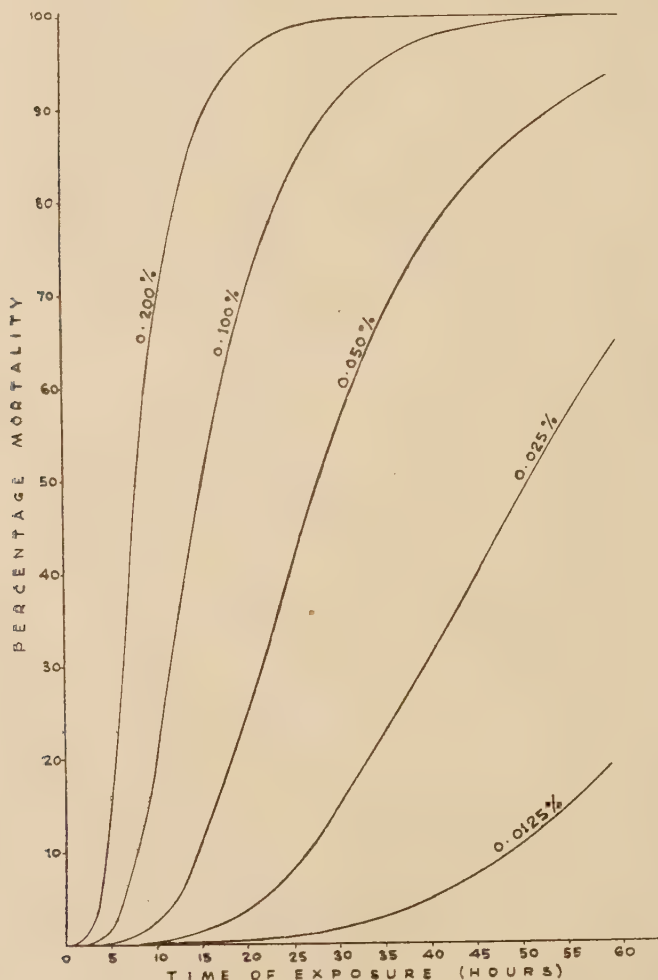
Fig. 1.—Mortality curves of *Macrosiphoniella sanborni* on chrysanthemum plants treated with various concentrations of 60·5 per cent. anhydride.

(*L. A. Lickerish*)

Dosage and Concentration.

The amount of bis(bis dimethylamino phosphonous)anhydride required to kill an insect when applied systemically varies from species to species even on the same plant. In general, larger species require a higher dosage. The following table gives the concentration which killed 50 per cent. of three species in 48 hours in laboratory tests.

	Per cent.
Macrosiphoniella sanborni on chrysanthemum ...	0·0165
Aphis fabae on beans	0·031
Pseudococcus citri on chrysanthemum	0·110
Pseudococcus citri on coleus	0·048

The speed of action is proportional to concentration or amount of insecticide applied to the plant. At the concentrations given in the above list of species that have been controlled, the kill was complete in about seven days. The best results were generally obtained by using 100 imperial gallons per acre on agricultural crops, but good results were obtained against *Myzus persicae* and *Aphis fabae* on sugar beet whether a given quantity of the anhydride was applied in 6 or 100 gallons per acre. On hops, 1½ pounds of Formulation 2 (66 per cent. anhydride) applied in 200 gallons per acre was found to be very effective, but the same quantity of insecticide in 60 gallons per acre was also effective.

Persistence of Insecticidal Activity.

Most sprayed crops remain toxic to Aphids for three to four weeks. Two applications, at an interval of four weeks, kept hops free of Aphids throughout the season, whereas hops sprayed with Parathion were reinfested by immigrant Aphids after two weeks. Strawberry aphis was controlled for a period of three to five weeks before a slight increase in population began ; in the same field, Parathion gave equally good control initially, but the Aphid population rose immediately at the same rate as on unsprayed plots. Groundnuts treated at a rate equivalent to ½ pound active material (100 per cent. anhydride) per acre remained toxic to Aphids for 24 days. When sugar beet was treated with Formulation 2 at a rate equivalent to 7½ pounds per acre it was found to be toxic to *Myzus persicae* and *Aphis fabae* for 14 days.

Behaviour in the Plant.

Translocation of the insecticide seems to be similar to that of food materials, the chemical being taken to areas of new growth ; thus, an excellent kill of blackfly on the seed head of sugar beet was obtained at the time of growth of the head. There is no kill below the level of spraying when only a certain part of the hop plant is sprayed, but there is a good kill above, even of immigrant Aphids that form colonies on leaves produced after the date of spraying. The insecticide is often much less effective on mature plants than on growing ones.

Phytotoxicity.

There is considerable variation in the concentration that causes damage to different plants. The following concentrations have been sprayed on the plants indicated, the figures being based on 30 per cent. anhydride. Where the concentration is known to be the highest at which no damage has occurred and higher concentration has caused damage, the figure is marked with an asterisk.

C

				Per cent.
Brussels Sprouts ...	...	...		10
Cabbage	...	...	...	5
Beet ...	...	...	...	5
Mangolds ...	...	...	...	15
Cineraria	...	...	...	1
Calceolaria	...	...	...	0·5*
Potatoes	...	...	...	0·3*
Chrysanthemum ...	...	...	...	0·5*
Carrots ...	...	...	...	0·5
Strawberries	...	...	...	1·0*
Blackcurrants	...	...	...	0·67*
Clover ...	...	...	...	1·0*
Roses ...	...	...	...	0·67* · (This concentration sometimes causes leaf-drop.)
Peas ...	...	...	...	0·6%

Apples :

Millers ...	...	...	0·6%
Worcester Pearmain		...	0·6%
Cox's Orange Pippin		...	0·51%* (Higher concentration causes leaf-drop.)

Compatibility.

The compound is not very reactive apart from hydrolysis, and even this is very slow under ordinary conditions. From the chemist's point of view, it would seem compatible at spray dilution with other compounds used in agricultural spraying (except, of course, with sulphuric acid). In particular, it can be used at spray dilution with any of the commercially available wetting agents. It is rapidly attacked by permanganate solutions, a property that can be made use of in cases of accidental ingestion. In the limited experiments on phytotoxic action carried out to date the compound has been found compatible with HETP, DDT wettable powder, copper oxychloride and cuprous oxide.

Action as Selective Insecticide.

Predacious insects are not killed by this insecticide, owing to the lack of contact effect. Adult Coccinellids and the larvae of Syrphids are unharmed by contact or by feeding upon Aphids that have died from the poison. This selective action is an added safeguard against the development of resistant races of pests. Larvae of *Pieris brassicae* are not affected by feeding on plants toxic to *Brevicoryne brassicae*.

Metcalf & March (1949) showed the anhydride to be completely inactive to bees (topical *$LD_{50} > 100$), cockroach and house-flies (topical $LD_{50} > 500$) and a very poor inhibition of bee brain cholinesterase (**$IN_{50} > 1·2 \times 10^{-3}$).

Application to plots or fields infested with cabbage aphis, strawberry aphis or blackfly resulted in prolonged control with only a slight increase in Aphid population

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*Median lethal dose by topical application in micrograms per gram body weight, assuming an average weight of 100 mg. per bee and 20 mg. per fly.

**Molar concentration for 50 per cent. inhibition of enzymes.

after three to five weeks. Fields sprayed, on the other hand, with Parathion and other similar general insecticides, such as HETP and Paraoxon,* showed a rapid increase in the pest a few days after spraying and the population built up within 10 to 14 days to a higher infestation than before treatment. Examination revealed that, on all fields sprayed with HETP, Parathion and Paraoxon, Syrphid larvae, Coccinellids and carnivorous Cecidomyiids, and also parasitic Hymenoptera of the genus *Aphidius*, were killed, while with the anhydride all these beneficial insects remained unharmed (figs. 2, 5, and 6).
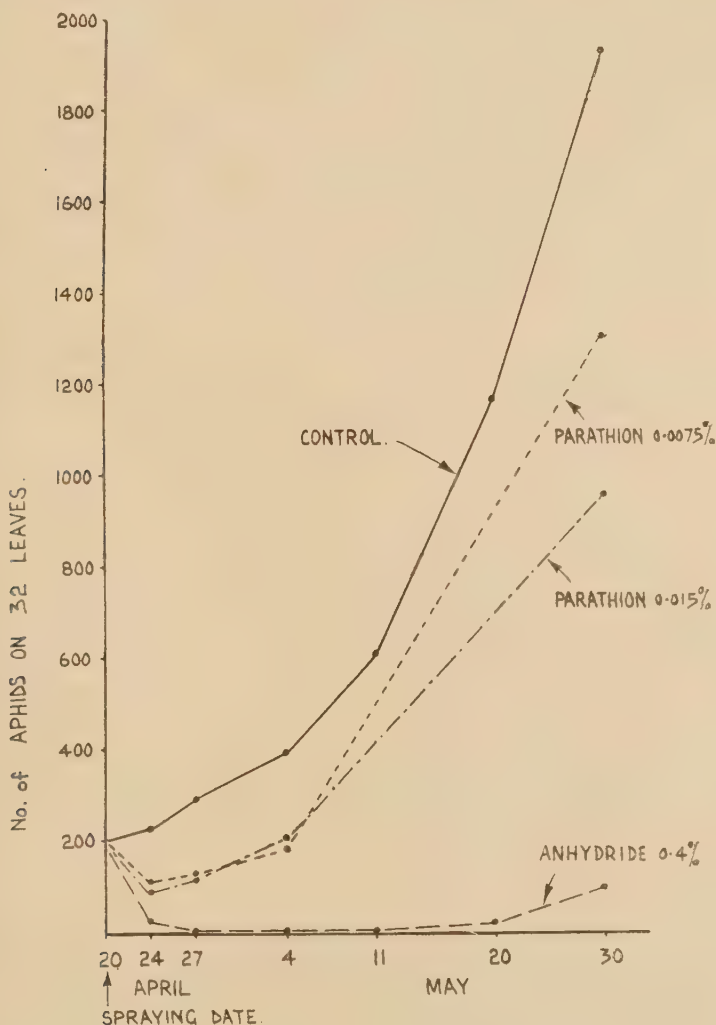
Fig. 2.—Population counts of strawberry aphis (*Capitophorus fragariae*) on replicated plot experiments after one spray application with each of two concentrations of Parathion and one application of the anhydride, with a hand sprayer.

(*D. W. Mollison*)

*Paranitrophenyl diethylphosphate.

Recommendation for Aphid Control.

The necessity for careful investigation of the insecticidal, phytopathological and medical properties of this chemical has resulted in a rather slow rate of progress and so far applications on a field scale have been confined to the following five problems.

Hop Aphid (Phorodon humuli).

Two applications of Formulation 2 to 1,500 acres of hops in Worcestershire, Herefordshire and Hampshire kept them free from Aphids and red spider and resulted in an increase of yield of from 2 to 4 cwt. per acre as compared with nicotine-dusted control plants. The first application ($\frac{3}{4}$ pound in 100 gallons of water per acre) was made at the end of May and beginning of June, before the Aphids appeared, and the second at the same concentration, but at 150 to 200 gallons per acre at the end of June and early July. Spraying should not be carried out later than six weeks before picking. In both cases, the spray was applied at 350 pounds pressure by means of a high-pressure, tractor-drawn, spraying machine fitted with whirl-disc nozzles. The spraying machine used was equipped with standard potato-spraying nozzles and pest guns. In these operations, the tractor was fitted with a cab in which the driver was protected from the spray and spray drift (Pls. VIII and IX). Operators were equipped with rubber gloves when handling the concentrate and wore white overalls which were frequently washed ; all work was carefully supervised.
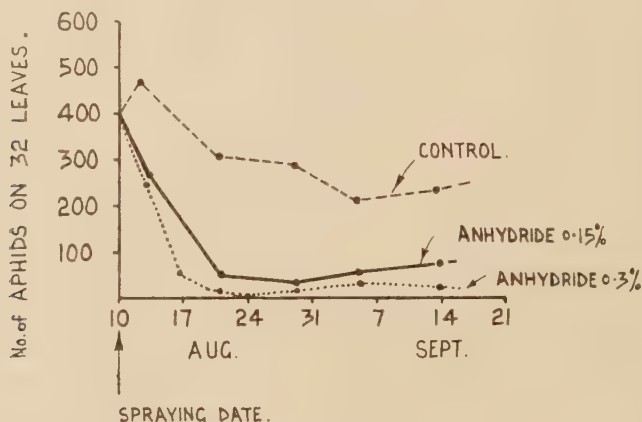
Fig. 3.—Population counts of strawberry aphis (*Capitophorus fragariae*) on replicated plot experiments after one spray application with each of two concentrations of the anhydride in the late summer, using a hand sprayer.

(*D. W. Mollison*)

Cabbage Aphid (Brevicoryne brassicae).

Good control of cabbage aphis was obtained in 1949 in fields of brussels sprouts in Bedfordshire by applying, with a high-pressure sprayer, 7 pounds of Formulation 2 in 100 gallons of water, with the addition of 6 ozs. Lissapol N, per acre. The spray was applied in mid-July or beginning of August and sampling was carried out in the following manner : Forty plants were taken from each test plot, twenty at equal intervals along each of the two diagonals of the plot commencing at a plant in the fourth row and 10 yards from the end of it. Two investigators worked each diagonal, taking 10 plants each in order to reduce the human factor.

The Aphid colonies were counted on all sample plants. Single Aphids were ignored and no attempt was made to distinguish between large and small colonies.
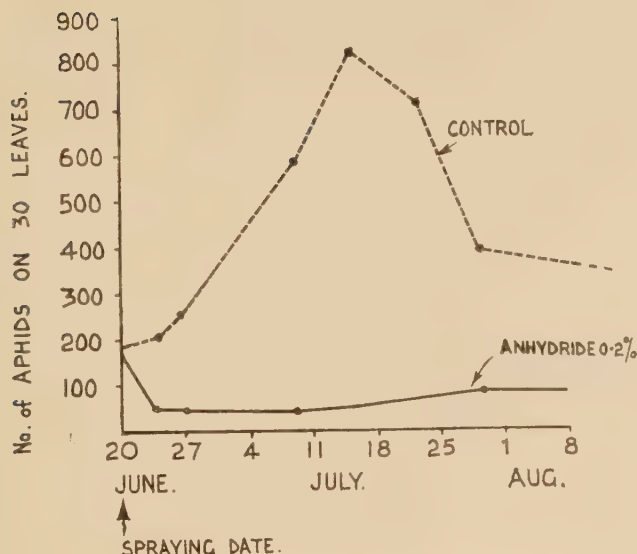
Fig. 4.—Population counts of strawberry aphis (*Capitophorus fragariae*) on a strawberry field which had been sprayed with the anhydride. Note the prolonged low level of Aphids. (*D. W. Mollison*)

In spite of an exceptionally severe outbreak the spraying was successful in keeping the Aphid population down for a period of seven weeks, during which time only very small colonies made their appearance. These were kept under control by the parasite, *Aphidius brassicae*, Marsh., Syrphid larvae and carnivorous Cecidomyiids. Unsprayed control plots were severely infested ; some plants were badly stunted and the sprouts of the early picking were all unsaleable because of Aphid contamination. Other plots sprayed with Parathion or Paraoxon required retreatment every 10 days, and, even so, did not produce such clean sprouts or such healthy plants as those treated with the anhydride. On many Parathion and Paraoxon plots, re-infestation occurred soon after spraying and built up to a far more serious infestation than was observed before (figs. 5 and 6). A high mortality of Syrphid and Coccinellid larvae was observed on the Paraoxon-sprayed plots and also, but to a less extent, on those sprayed with Parathion. Spraying machines were equipped with gas proof cabs ot the kind used for dinitro-orthocresol and Parathion spraying (see Pls. VIII and IX).

As in the case of hops, spraying should not be carried out later than six weeks before picking.

Strawberry Aphid (*Capitophorus fragariae*).

Replicated plots were sprayed with the anhydride, Parathion and Paraoxon, by means of a hand sprayer. The Aphid population was sampled by selecting 16 plants at one-yard intervals in each row of each plot, choosing on each plant the leaf that was the nearest to being full grown but was not yet opened and counting the Aphids present thereon (figs. 2 and 3).

These results were verified on a field scale (fig. 4). From these and other experiments, it was concluded that 2 pounds of Formulation 2 in 100 gallons, applied at the rate of 100 gallons per acre during the pre-flowering period, kept the plants free from Aphids until the fruit was picked. The fields were then sprayed again, as soon

as the Aphids made their appearance after the picking of the fruit. In the case of strawberries, it is recommended that no spraying should be carried out later than four weeks before picking.
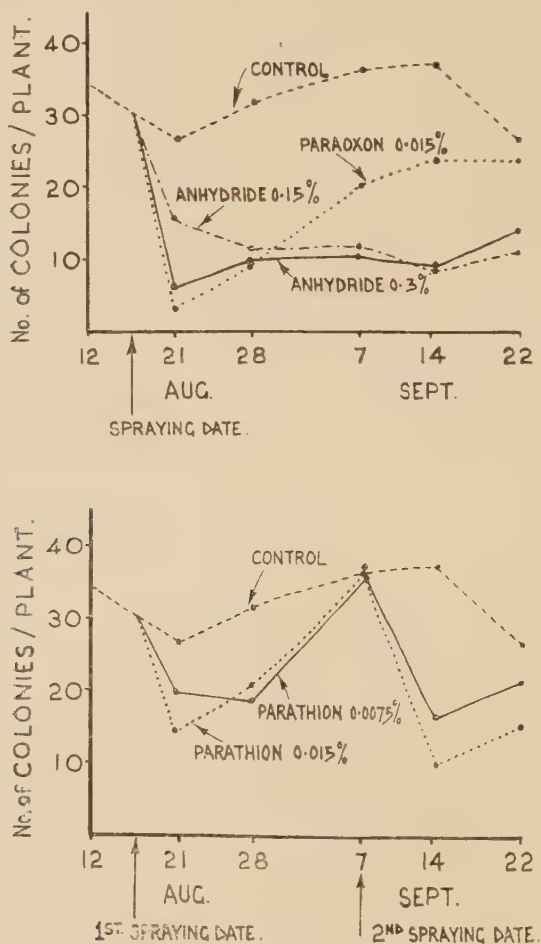
Fig. 5.—Population density of cabbage aphis (*Brevicoryne brassicae*) on brussels sprouts plots sprayed with Parathion, Paraoxon and the anhydride. *The upper graph* compares the prolonged low population density after application of the anhydride, with the rapid build-up after Paraoxon application. (The spray chemicals were applied on August 16th.) *The lower graph* shows the population density of cabbage aphis after two Parathion sprays ; the second one was necessitated by the rapid build-up of the cabbage aphis after the first spraying.   (*J. P. Tunstall*)

If control of the strawberry aphis is to be combined with the prevention of the virus diseases, yellow edge and crinkle, then it is necessary to spray before the Aphids make their appearance, and in any case not later than within four weeks of fruiting. It is recommended that as soon as the fruit has been picked, the plants should be re-sprayed. Maidens should be sprayed as a routine measure in mid-May and again at the end of June if the treatment is intended to prevent the spread of virus diseases. Treatment of maidens of plants obtained from virus-free stock will go far towards preventing losses of susceptible varieties such as " Royal Sovereign ".
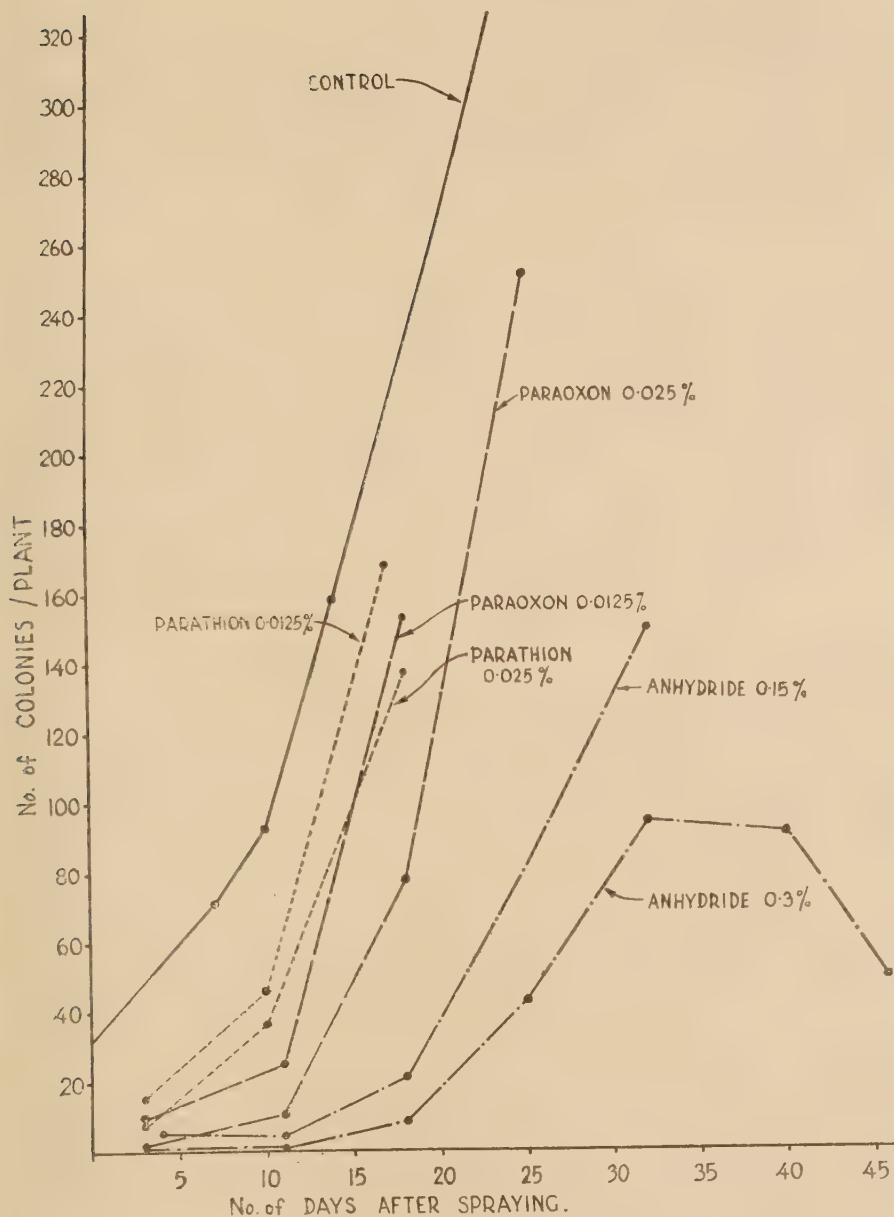
Fig. 6.—Population density of cabbage aphis (*Brevicoryne brassicae*) on brussels sprouts measured by the average number of colonies per plant during a period of a few weeks after the application of Paraoxon, Parathion and the anhydride. It will be noted that the increase in population after Paraoxon and Parathion spraying is much more rapid than after spraying with the anhydride. With the latter, good control was effected for about 16 to 18 days during September, 1949, when the weather conditions were very favourable to cabbage aphis. (*E. G. Goscombe*)

Rose Aphid (Macrosiphum rosae).

It has been found in experiments, and on a field scale, that the application of Formulation 2 to roses, as soon as the Aphids make their appearance, at a concentration of 1 pound in 100 gallons frees the roses from green fly within a week and keeps them free for three to six weeks. In experimental work and applications on a garden scale, no marking of the leaves or of the blooms has been observed. Any small garden sprayer can be used. Nurseries have been sprayed with a machine mounted on a 4-ft. high-clearance tractor.

Tobacco Aphid (Myzus persicae).

It has been found in Rhodesia that the treatment of tobacco plants with $7\frac{1}{2}$ pounds of Formulation 2 in 100 gallons of water, applied at the rate of 100 gallons per acre, gives excellent and prolonged control of green fly. This treatment should be carried out when the first Aphids appear and no application should be made later than six weeks before harvesting the leaf.

The work done on the control of the numerous other pests against which promising results have been obtained, such as Aphids on chrysanthemum and cineraria, woolly aphis on apples, red spider on various fruit trees and *Aphis laburni* on groundnuts in Rhodesia and East Africa is not yet sufficient to permit of any general recommendations. Research on the control of Aphids and red spider is being continued and a further report will be made at a later date.

Control of Virus Diseases.

An insecticide that will give plants a prolonged toxicity to Aphids suggests the possibility of controlling virus diseases through control of their vectors. With this in view, field experiments have been carried out against the sugar-beet yellow virus transmitted by *Myzus persicae* and *Aphis fabae* (figs. 7 and 8), and preliminary results in 1948 showed a reduction in infection from 68 per cent. of the plants on unsprayed plots to 13 per cent. on sprayed plots. Even in 1949, when a very severe attack of *Myzus persicae* and *Aphis fabae* was experienced in East Anglia, the virus incidence on sprayed plants was reduced to less than half and the outbreak of the disease held in check for a period sufficient to produce a significant difference in the colour of the leaves in September and a substantial improvement in yield and sugar content. In a similar way, a reduction in the yellow-virus incidence on sugar-beet stecklings was obtained with several treatments in the autumn, resulting in a decrease in infection from 100 per cent. in the unsprayed steckling beds to a very small percentage in the sprayed beds.

Toxicity to Animals.

The toxic symptoms are similar to those caused by other organic phosphorus compounds at similar sites of action. The toxicity, however, is lower and the symptoms are less marked than with the better known insecticides, HETP, TEPP, Parathion and Paraoxon. The cholinesterase inhibition by these last named substances is very spectacular. R. L. Metcalf, in a lecture entitled "Insect toxicological Studies with new organic compounds," delivered at the 61st Annual Meeting of the American Association of Economic Entomologists in December, 1949, stated that the anhydride was a very poor inhibitor of the cholinesterase of mammals.

The lethal dose was established on the following animals, the poison being introduced by various methods. Incidentally Metcalf, in the lecture referred to above, reported the LD_{50} for the mouse to be 17–18 mg. per kg.

Animal	Method of entry	Lethal dose
		(mg. per kg. body weight)
Guineapig	Peroral with bran	22
Rat	Peroral, using feeding tube	18
Rat	Percutaneous alcohol solution rubbed	
	on shaved skin	200
Rat	Subcutaneous injection	18
Dog	Peroral with feeding tube	More than 10
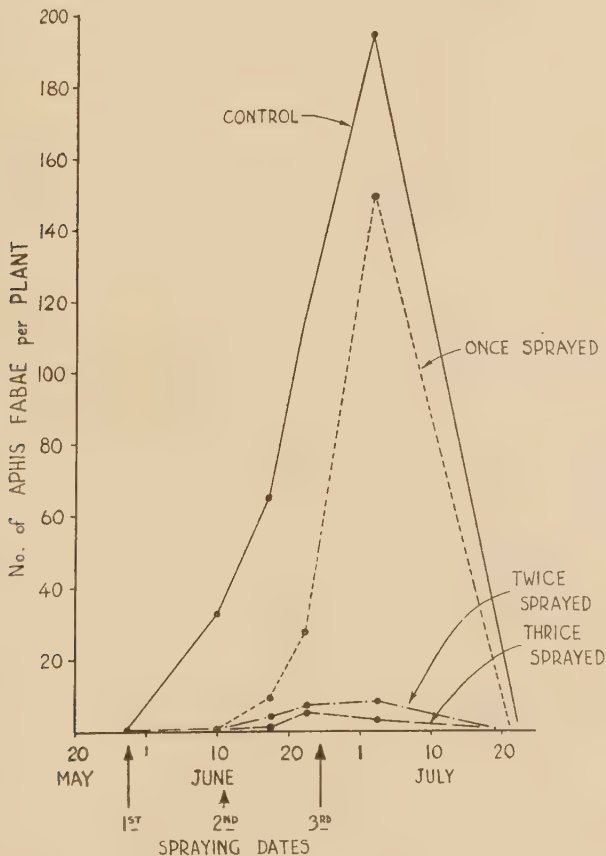
Fig. 7.—Population density of *Aphis fabae* in a sugar-beet field at the Norfolk Experiment Station, Sprowston, sprayed once, twice and three times with 5 pounds of the anhydride in 100 gallons of water per acre.

(*J. W. Cowland and W. Heatherington*)

Symptoms of poisoning.

The pathological symptoms of acute poisoning, as produced by lethal and slightly sub-lethal doses, vary with the species of animal examined. They usually include salivation, foaming at the mouth and nose, nausea and vomiting, lockjaw (in rats), watery bowels, tremors, twitching of all muscles, bristling of the hair, staring

protrusion of the eyes, stiffness and paralysis of the hind legs, deep and frequent respiration, bradycardia and lowering of blood pressure. Death occurs sometimes under symptoms of respiratory failure.
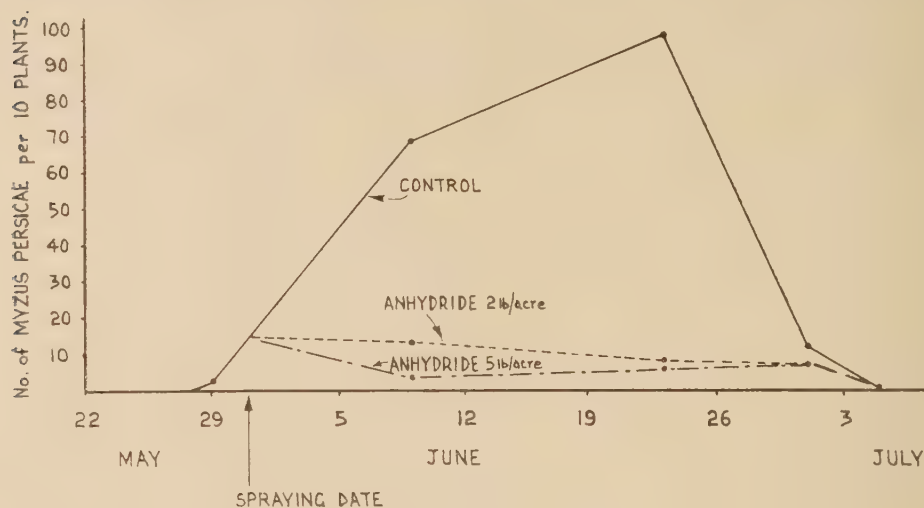
Fig. 8.—Population density of *Myzus persicae* on a sugar-beet field at Docking sprayed once only with 5 pounds of the anhydride in 100 gallons of water per acre.

(J. W. Cowland and W. Heatherington)

An histological examination of the animals killed revealed very considerable hyperaemia in all organs, especially in the lungs, brain and intestines. Other symptoms observed were necroses of the mucosa in the whole intestinal tract, especially in the colon, bleeding in the lungs, such as occurs in a haemorrhageous pneumonia, degeneration of some ganglion cells in the brain, high oedema and localised bleeding in the cortex, bleeding, seric oedema and fatty degeneration of the cells in the liver, and hyperaemia with degeneration of the tubuli in the kidneys.

Small sub-lethal doses have been given at frequent intervals over prolonged periods without any evidence of cumulative effects. Dogs fed daily with a fifteenth of a lethal dose for three weeks showed, after that period, no signs of pathological symptoms, apart from a slight degree of hyperaemia of the tissues. Guineapigs rubbed daily on shaved areas of the skin with an alcoholic solution of the chemical so that 1 mg. per kg. body weight was applied daily for three weeks appeared absolutely healthy after that period, but histological examination revealed slight fatty infiltration of the liver as well as moderate hyperaemia.

Early symptoms of poisoning after a sub-lethal application are protruding eyes, lachrymation, salivation, hyperaemic conjunctiva and diarrhoea.

Treatment of poisoning.

In cases of acute poisoning of rats and dogs, injections of considerable quantities of atropine (0·2 mg. per kg. in the case of dogs, 2 mg. in the case of rats) caused the disappearance of all pathological symptoms except tremors and twitching, which were somewhat reduced but did not disappear. Dogs treated with lethal dosages of the anhydride, but saved by atropine treatment, showed symptoms of nervous disorders (middle brain symptoms). These were remedied by subcutaneous injection of Vitamin B 1, in quantities of 5 mg. per kg.

When a 0·2 per cent. solution of the anhydride, to which an equal volume of a 0·1 per cent. solution of potassium permanganate had been added was fed, after standing for some minutes, to the animals by a feeding tube, the toxicity was decreased to one-half. It was possible to save animals that had received a lethal dose, by giving 0·1 per cent. potassium permanganate at any time between a few minutes and half an hour after the taking of the poison, but no regular correlation between the timing of this treatment after feeding, and the degree of recovery could be established, possibly owing to individual differences in the state and reaction of the digestive tract.

Feeding trials with sprayed plants.

Extensive trials with rabbits and guineapigs were carried out for prolonged periods with plant material that had previously been sprayed.

Peas.

(a) Peas sprayed against green fly on the 28th July at the rate of 3 pounds of Formulation 2 per acre and picked on the 8th, 13th and 20th August were fed to rabbits for a period of 22 days at the rate of 2 ounces of shelled peas per animal each day, so that the total intake per rabbit during the course of the experiment was $2\frac{3}{4}$ pounds. The daily intake of each rabbit represents the equivalent of $5\frac{1}{2}$ pounds of peas for a man weighing 70 kgs.

(b) In a further experiment, 4 ounces of pea shells were fed daily for 22 days ; in this case the peas had been sprayed on the same date as in experiment (a), but with 6 pounds, not 3 pounds, per acre. The total intake per rabbit during the course of the experiment was $5\frac{1}{2}$ pounds.

No abnormality or toxic symptom of any kind was observed in either of these experiments and no pathological symptoms were found in the histological examination of the organs.

Strawberries.

Strawberry plots were sprayed with $1\frac{1}{2}$ pounds of Formulation 2 per acre, 1, 2, 3 or 4 weeks before picking. The strawberries were kept in cold storage after picking and then warmed out and fed raw to guineapigs at the rate of 2 ounces per day for 20 days. Controls were fed on untreated strawberries picked at the same time and kept in cold storage.

The intake per guineapig during the course of the experiment was $2\frac{1}{2}$ pounds and the daily intake of each represented the equivalent of 10 pounds for a man weighing 70 kgs.

No ill effects were observed and the animals behaved normally and remained in good condition, with healthy appetites. Although special attention was paid to the possibility of gastro-intestinal or central-nervous-system disturbances, all observations were negative, and there was none of the lachrymal symptoms that are observed even in mild toxic action. No pathological symptoms were found in the histological examination of the organs. The controls differed in no respect from the animals fed on treated fruit.

Apples.

Apples sprayed approximately 1 month before picking were fed to rabbits at the rate of 4 ounces per day for 56 days, each animal taking 14 pounds during the period.

No toxic symptoms whatsoever were observed. The daily intake is equivalent to a consumption of 14 pounds of apples in a normal man weighing 70 kgs.

Hops.

(*a*) A sample of hops from the field that had had a second application of $1\frac{1}{2}$ pounds of Formulation 2 per acre 6 weeks before picking, was treated with 10 times its weight of boiling water for 2 hours. The extract was filtered and after cooling added to 8 times its own volume of water. This infusion was fed to guineapigs at the rate of 10 cc. per day in bran and oats, the amount of the infusion given being equal in hop content to $2\frac{1}{2}$ pints of pre-war beer per day for a man weighing 70 kgs. The infusion was made to the highest figure given by Thorpe (Dictionary of Applied Chemistry), which is approximately four times that of present-day beer. Consequently, the amount of hop extract fed would be equivalent to 10 or more pints of present-day beer per day for a man weighing 70 kgs.

No ill effects were observed, even though particular attention was paid to the possibility of gastro-intestinal, central-nervous-system or lachrymal disturbances. All the guineapigs were in excellent condition ; two sows gave birth to young during the experiment and continued with their experimental feeds while suckling their young. The animals appeared in every respect as normal as those in the breeding pens, and were very lively when put into the exercise pen. They were fed for 4 weeks and the experiment is still continuing at the time of writing.

(*b*) A similar experiment was carried out with rabbits. A hop infusion, prepared as in the experiment with guineapigs from hops similarly treated, was fed for 4 weeks to the animals at the rate of 30 cc. per day. The amount given was equal to the hop content of $7\frac{1}{2}$ pints of pre-war beer per day for a man weighing 70 kgs., or 30 or more pints of present-day beer.

No ill effects whatsoever were observed and no pathological symptoms were found in the histological examination of the organs.

(*c*) A further experiment was carried out with hops deliberately sprayed only one week before picking with 1 pound of Formulation 2 per 100 gallons per acre. The dried hops were treated with ten times their weight of boiling water for 2 hours and the extract then filtered. Guineapigs were fed with this concentrated infusion, 6 cc. per animal in one batch and 12 cc. in a second, with bran and oats in both cases. The amounts of hops fed in this experiment were equivalent to 12 and 24 pints of pre-war beer for a man weighing 70 kgs. or 48 and 96 pints of present-day beer.

As was the case in previous experiments, the animals took the treated food with normal appetite, and there were no symptoms of any physical change, nor were there the slightest signs or symptoms of gastro-intestinal, central-nervous-system or lachrymal disturbances. No pathological symptoms were found in the histological examination of the organs.

In spite of these experiments, it is considered prudent to recommend that hops should not be sprayed later than 6 weeks before harvesting.

It will be seen that none of the sprayed products fed to animals produced any toxic effect. Similar results were obtained in 1948 when plants, that had previously been sprayed, were fed to animals. The following table gives a summary of the results. In no case was any toxic symptom observed.

	Dosages (in pounds/acre) of 66 per cent. anhydride	Quantity consumed per animal (pounds)	Period between spraying and feeding
Sugar beet	15	12¾	10 weeks
Brussels sprouts	1½	20	10 days
Brussels sprouts	3	20	10 days
Pea plants	1	16	30 days
Pea plants	2	16	30 days
Pea pods and peas	1	6¾	60 days
Pea pods and peas	2	6¾	60 days

Precautions for Use in the Field.

This insecticide should be treated with the same respect as nicotine and all contact with the concentrate should be avoided. Where accidental splashing of the skin or clothes occurs, the concentrate or dilute solution should be washed off first with water and then with 1 per cent. sodium permanganate solution. Protective cabs should be used to prevent spray drift from reaching the operator. Rubber gloves should be worn when the chemical is being mixed and overalls should be worn during work and then removed and frequently washed. Face and neck masks should be worn when a protective cab cannot be provided. Spraying machines should be well hosed down before they go into the workshops for repair. The work people should be under constant medical supervision, and a protein-rich diet, e.g. 2 pints of milk daily, should be consumed by operators working with the chemical. Great caution is essential despite the fact that the chemical is less dangerous than Parathion or HETP.

The precautions to be taken in the field are set out below. Although nearly 2,000 acres have been sprayed in England in 1949, there has not been a single casualty. Any recommendations for treatments are, therefore, based solely on animal experiments.

1. In cases where the poison is taken up orally, an extensive gastric lavage with 0·1 per cent. potassium permanganate solution should be carried out. Later, the stomach should be washed with dextrose solutions (20 per cent.).

2. When vomiting or other acute symptoms of poisoning appear, atropine should be injected in high dosages, commencing with 1 mg. per injection. Repeated enemas with a charcoal slurry might be useful.

3. Where nervous disorders appear, Vitamin B1 should be given in dosages of 2×100 mg. per day.

Chemical Determination in Plants.

Principle of Method.

The compound in its crude form is a viscous liquid completely miscible with water, but it has been obtained as a solid with a melting point *circa* 20°C. Between water and immiscible organic solvents, except chloroform, it partitions heavily in favour of water. In dilute solution the partition between chloroform and water is 7 : 1 in favour of chloroform.

No naturally occurring phosphorus compounds are soluble in chloroform with the exception of fatty phosphatides such as lecithin, from which the phosphate is readily split off by alkaline hydrolysis. The anhydride is very stable in alkaline solution, there being only about 1 per cent. decomposition in one hour at 80°C. in N/10 alkali, but it is completely hydrolysed in 1N. strong acid at 100°C. in 30 minutes.

The method of determination in plant or animal extracts is based on these facts. Compounds hydrolysable in alkali are removed from a chloroform extract which is then evaporated and the residue hydrolysed by acid. Standard methods are employed to determine the dimethylamine or phosphate content, or both, of the hydrolysed residue. If phosphate is to be determined, it is necessary to make certain that the chloroform residue is fully oxidised by digestion with perchloric acid, excess of which is subsequently evaporated . The most sensitive method is the molybdenum-blue colorimetric method for phosphate.

The impurities associated with the compound are amine phosphates derived from hydrolysis of less stable partial amination products and tris dimethylamino phosphine oxide, $(Me_2N)_3PO$. The former are not chloroform-soluble, but the latter is very stable to alkali, extractable from water with chloroform and hydrolysed by acid. It will, therefore, register in the analysis as bis(bis dimethylamino phosphonous) anhydride if phosphate determination only is used, but this will give the result an error on the side of safety. It is of no importance for insect control, is probably less toxic to mammals than the anhydride and will be largely lost by evaporation.

Method of Estimation.

One kg. of shredded plants is macerated with 1,000 cc. of water and sufficient caustic soda added to bring the alkalinity to N/10. The broth is heated to 80°C. for 30 minutes (after which time hydrolysis of lecithin-type compounds will be complete) ; it is then strained and the liquor is further clarified by filtration, a standard kieselguhr filter aid being used if necessary. An aliquot portion of the filtrate is extracted once with its own volume of chloroform. The extract is distilled until 90 per cent. of the chloroform is removed and the residue is refluxed with its own volume of 10 per cent. hydrochloric acid for 30 minutes. The rest of the chloroform is then distilled off. The residue or an aliquot portion thereof is evaporated to dryness, treated with a few drops of perchloric acid and again evaporated to dryness. A phosphate estimation is carried out on the dried residue by the molybdenum-blue method as improved by Allen (1940). The bis(bis dimethylamino phosphonous)anhydride is determined from the relationship 4·6 mg. of the anhydride = 1 mg. Phosphorus. Add 14 per cent. for incomplete extraction.

Very tedious liquid and solid separations may be involved in extractions of vegetable or animal matter. It is not, however, of importance to separate more than an aliquot portion of clear chloroform if the method of single extraction with chloroform is used. Similarly, it is not essential to remove solid matter from all the original liquid extract, if the liquid content of residue is also determined by drying a portion of it.

Accurate results can be obtained with the molybdenum-blue method for phosphorus on 0·1 mg. of phosphorus (equivalent to 0·46 mg. of the compound). One hundredth of this quantity can be detected.

A blank test should, of course, be made on similar unsprayed vegetable matter to allow for the effect of any interfering substances. Such interference has not so far been met.

Analyses carried out on Hops and Sugar Beet.

Although previous experiments had shown no trace of the insecticide on sprayed hops at the time of harvesting, further samples from each sprayed hop garden were collected and analysed by the above method. No trace of the anhydride could be detected. Similar experiments have been carried out during the past two years on sugar beet which had been treated for the control of yellows and again no trace of the bis(bis dimethylamino phosphonous)anhydride could be found at the time

of harvesting. The same remarks apply to strawberries which were treated while budding ; the fruit was harvested and analysed without any trace of the compound being detected.

In addition, some hops were sprayed, intentionally, only one week before harvesting. The infusion made from these hops was examined by the above method but no trace of the chemical was found. This result agrees with the results of the animal experiments reported on p. 496.

A sample of bis(bis dimethylamino phosphonous)anhydride has been made from the radioactive P³²phosphorus isotope, but results of investigations into the distribution and fate of the resulting compound in the plants are not yet available for publication.

Summary.

Bis(bis dimethylamino phosphonous)anhydride is shown to be a systemic insecticide when sprayed on the leaves of numerous plants. The translation of it from one part of the plant to another, over distances varying from the thickness of a leaf to three feet, was shown with *Aphis fabae*, *Myzus persicae*, *Brevicoryne brassicae*, *Macrosiphoniella sanborni* and *Pseudococcus citri*.

Plants treated with it are shown to be toxic to 14 Aphid species, one Aleurodid, one mealy-bug, two Jassids and two species of red spider.

It is not found to be toxic to non-plant-sucking insects, notably predators and parasites. It is, therefore, a selective insecticide for the control of Aphids, and field experiments have shown that treatment with it gives plants prolonged toxicity to Aphids and allows the parasites and predators to keep in check any survivors or newly arrived individuals.

Non-selective organic phosphorus insecticides such as Parathion, Paraoxon and HETP give a high mortality, but the Aphid population builds up again very rapidly after treatment with them, leading, in many cases, to a heavier infestation than before.

Plants treated with bis(bis dimethylamino phosphonous)anhydride, on the other hand, keep free from Aphids for prolonged periods (2 to 5 weeks depending on the species of Aphid, the stage of growth of the plant and its physiological condition).

Chemical and physical properties and formulations are reported.

Extensive field experiments have proved that a formulation containing 66 per cent. bis(bis dimethylamino phosphonous)anhydride is a valuable insecticide for the control of Aphids and red spider :—

(*a*) Two applications of $\frac{3}{4}$ pound in 100 gallons of water per acre kept hops free from red spider and Aphids during the growing season under English climatic conditions.

(*b*) An application of 7 pounds in 100 gallons per acre against *Brevicoryne brassicae* on cabbage and brussels sprouts kept the plants practically free from Aphids for a period of over five weeks during the very severe cabbage-aphis outbreak of 1949.

(*c*) On strawberries, two applications of $1\frac{1}{2}$ pounds in 100 gallons per acre kept the plants free from the strawberry aphis (*Capitophorus fragariae*).

(*d*) On roses, one spray of 1 pound in 100 gallons effected satisfactory control of the rose aphis (*Macrosiphum rosae*).

(*e*) Seven and a half pounds in 100 gallons per acre effected practical control for three weeks of *Myzus persicae* on tobacco in Rhodesia.

(*f*) Repeated applications on sugar beet reduced the population of *Myzus persicae* and *Aphis fabae* considerably and also retarded and reduced the yellow virus infection.

The toxicity to mammals was explored by introducing the poison by various methods. The lethal dose by peroral application was found to be 18 mg. per kg. body weight for the rat and 22 mg. per kg. for the guineapig. The lethal dose by subcutaneous injection for the rat was 18 mg. per kg. and, by percutaneous application, 200 mg. per kg. The clinical features of the toxic symptoms are described and in addition the results of histological examination. The anhydride is not as toxic as Parathion, Paraoxon or HETP but it is nevertheless a very toxic compound. Suggestions for the treatment of casualties, based on animal experiments, are reported.

Feeding trials with treated plant material were carried out. Animals were fed at repeated intervals with shelled peas, strawberries, apples and hops obtained from crops that had been sprayed during the growing season. Much larger quantities were fed than could be expected in the case of human consumption of such food, and in no case were any toxic symptoms observed nor did histological examination of the organs of the animals reveal any pathological phenomena or any hyperaemia.

Precautions for use in the field should include the use of protective cabs on tractors to prevent the spray drift from reaching the operator and the wearing, by persons handling the concentrate, of overalls and rubber gloves to avoid skin contact.

A method of analysis for bis(bis dimethylamino phosphonous)anhydride in plant material is described ; it is sensitive to 0·46 mg. of the compound. Sugar beet and hops that had received spray treatment during the summer were examined by this method at harvest time and no trace of the systemic insecticide was found to be present in the plants.

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Tractor-driven high-pressure sprayer taking in spray liquid from a bulk handling unit. The tractor is provided with a gas-proof cab; the chimney-like charcoal filter on the top of the cab and the adjacent electrically driven fan will be noted. The operators wear white overalls and rubber gloves whilst handling the concentrated liquid. On the supply tanker, calibrating vessels are fitted, into which the liquid is pumped, avoiding the handling of tins and the likelihood of splash.

A side-view of the protective cab showing charcoal filter and electric fan, also rubber beading on the door to effect a seal.

MOSQUITO CONTROL: AN INVESTIGATION OF NATURAL SURFACE FILMS IN RELATION TO THE SPREADING OF LARVICIDAL OILS UPON WATER.

By B. A. Toms, D.Sc.

Courtaulds Limited Research Laboratory, Maidenhead, Berks.

A necessary quality of larvicidal oils is that they should spread upon natural water surfaces, and it is largely by virtue of this property that adequate reduction and control of mosquito breeding can be achieved over large areas with economy of oil and of effort.

Oils differ in their ability to spread upon water and for this reason users of large amounts of larvicidal oil try to obtain one that is likely to meet their particular needs. The choice is usually made by observing the behaviour of samples of the available oils in empirical tests of spreadability. Sometimes this testing involves no more than rough comparison of the rate and extent of the spreading which occurs when single drops of the different oils are placed in turn upon the (clean) surface of water. Alternatively, the spreading pressure of each oil is compared with that of a "standard" specimen of, say, castor oil (Murray, 1939). Both methods are widely used, yet neither of them, as ordinarily practised, can provide the information sought since almost no account is taken of the variety of waters to be oiled, even in a small control area. It often happens, therefore, that oil which spreads well at some sites will fail to spread at others where the water surface has different properties. Moreover, because the properties of an exposed water surface can change, considerable variations in spreading may be observed when portions of the same batch of oil are applied at one site but at different times.

For these reasons, and because it is possible to increase the spreading power of an oil by adding to it quite small amounts of "spread-aiding" substances, it would clearly be advantageous to be able to arrange, before distribution begins, that a larvicidal oil has optimum spreading properties in relation to the conditions prevailing in a particular area at a particular time. The purpose of this paper is to describe how this was done in a part of the Gold Coast in 1944. As well as explaining the method, and recording data, the account will also serve to indicate that the requisite survey of a large area of country can be undertaken by a solitary trained observer with very little equipment.

Method of studying Films on the Surface of Water.

Natural surface films are thin membranes which form when exposed waters are undisturbed by wind or rain. These films may be visible or invisible and the matrix, or foundation, of most of them is thought to be a bacterial slime. In this are embedded certain other materials which may be classified roughly as "biological" or "mineral" according to their nature. In the former group are bacteria, protozoa, algae and pollen; "mineral" contaminants include dusts and inorganic colloids, often of a ferruginous nature (Williamson, 1944). Although a light breeze can cause them to move to the leeward margins of the water surface, these natural films adhere to the surface and possess a certain rigidity so that they resist displacement and lateral compression by small forces.

When considering the spreading of oil upon a natural water surface it is convenient to imagine that the size of the interface produced depends on two factors. On the one hand there is the intrinsic spreading power of the oil: this is measured by its

spreading pressure, which is the force, in dynes per centimetre, that must be opposed to the advancing edge of a drop, just to prevent it from spreading on a clean water surface. On the other hand there is what we shall term the " resistance " of the natural surface film which hampers the spreading of oil : this is measured by the *film pressure*, a force equal in magnitude to the spreading pressure of an oil which just fails to spread on the contaminated surface.

It follows that in principle there are two methods by which the suitability of a larvicidal oil can be assessed from the spreading standpoint :—

(i) By determining the frequency of spreading when drops are placed on a large selection of the accessible water surfaces.

(ii) By determining the resistance of the films on a large selection of the accessible water surfaces.

Of these, (ii) is clearly to be preferred because if spread-aiders are available it should be possible to make an oil which could be relied upon to spread at most of the known breeding places.

A simple, yet accurate and reliable method for measuring the resistance of surface films was described by Adam (1937) and has since been used in the field by Renn (1942). Briefly, a series of " spreading standards ", *i.e.*, liquids of known and constant spreading pressure, is obtained. At first, Adam (*loc. cit.*) employed standard solutions of lauryl alcohol, $C_{12}H_{25}OH$, in a water-white mineral oil, but more recently (1945) he has described two other series of spreading standards, one derived from oleyl alcohol, $C_{18}H_{35}OH$, and Liquid (" Medicinal ") Paraffin, and the other consisting of mixtures of terpineol and the same non-spreading diluent. The spreading pressures of these new standards are independent of the normal variations of the temperature and acidity of natural waters, and the concentrations of the solutions can be chosen so that there is a convenient difference (e.g. 5 dynes/cm.) between the spreading pressures of adjacent members of each series.

A determination of the film pressure on a natural water surface, or a part of it, is begun by placing a drop of the standard solution having the lowest spreading pressure on the water. If this shows no tendency to spread a drop of the next (higher) member of the series is placed nearby and its behaviour observed. This orderly procedure is continued until a solution is found which can just displace, or push aside, the natural film. The appropriate film pressure then lies between the spreading pressure of this standard solution and that of the preceding, lower member of the series which just failed to spread.

The writer prepared two series of spreading standards using the following materials :

(i) Oleyl Alcohol (Iodine value=61·8 ; Saponification value=0·5 mg. KOH per g.)

(ii) Terpineol

(iii) Liquid (" Medicinal ") Paraffin (Specific gravity=0·846).

The compositions of the liquids, and their spreading pressures, are set forth in Tables I and II.

The two first mentioned were obtained from Prof. N. K. Adam, F.R.S., and were samples of the materials which have since been described by him (1945). Their spreading characteristics were therefore accurately known, and the spreading pressures quoted in Tables I and II are nominal values deduced by graphical interpolation of his data.

TABLE I.

Spreading Standards, Series A : Mixtures of Oleyl Alcohol and Liquid Paraffin.

Mixture No.	g. Oleyl alcohol per 100 ml.	Spreading Pressure (dynes/cm. at 20°C.)
1	0·15	10
2	0·35	15
3	0·60	20
4	1·0	25
5	2·0	30
6	3·9	35
7	6·5	40
8	10·0	45

TABLE II.

Spreading Standards, Series B : Mixtures of Terpineol and Liquid Paraffin.

Mixture No.	ml. Terpineol per 100 ml.	Spreading pressure (dynes/cm. at 20°C.)
1	0·10	5
2	0·30	10
3	0·65	15
4	1·5	20
5	5·0	25
6	10·0	30
7	Terpineol, pure	36

By means of these spreading standards the film pressures obtaining on natural waters could be determined and classed in the ranges 0–5, 5–10, 10–15,. . . 40–45, and greater than 45 dynes/cm.   Thus, the resistance of a film allocated to the " 30–35 " class was such that Mixture A5 (Table I, no. 5) just failed to spread while Mixture A6 displaced it (perhaps very slowly).

For use in the field the spreading standards were stored in flat 1 oz. brown glass bottles each fitted with a cork in which was inserted a piece of thin glass rod : this rod dipped into the liquid and served to deliver single drops of solution when removed and shaken.   The bottles were carried in a small wooden case with partitions inside to keep them upright and apart.

Investigation of the Films on natural Waters in the Gold Coast.

This investigation was carried out from September to November 1944 in that part of the Gold Coast delimited by the coastal towns of Sekondi (4° 56′ N, 1° 43′ W) and Axim (4° 52′ N, 2° 14′ W) and extending inland for a distance of about 15 miles. This region has an equatorial climate and much of the land is covered with tropical rain-forest. Parts are permanently waterlogged or liable to be flooded after heavy rain. At the time of the experiments the countryside was drying with the onset of the annual dry season, which lasts from November to April, and much casual water left by the rains of May, June and July had already disappeared.   Nevertheless an interesting selection of sites remained, and these were augmented, from time to time, by heavy showers of rain.

The waters examined included rain-pools in forest tracks, shallow waterlogged irrigation and roadside ditches, borrow pits, ponds, streams, river margins, and both temporary and permanent swamps.   The sites were selected at random.

It soon became apparent that, from the physical standpoint, the films under investigation were of two types which may be distinguished as Uniform and Non-Uniform. When a uniform film was present, very nearly the same film pressure was recorded in all parts of the water surface. But if the film was of the non-uniform type, considerable differences were observed among the pressures determined in different places on the surface even though these were sometimes no more than 6 to 12 inches apart.

Substantially uniform films were detected at all sites where the water surface could be described as " clean " (i.e. free from visible scum when examined by reflected light) even though the water might be turbid. Non-uniform films were frequently, if not always, found where a surface scum of some kind was clearly to be seen. It was of particular interest and importance to find that if a part of a water surface was separated from the rest by some natural barrier (floating twigs, débris, weeds, mud) films having quite different properties could exist on either side of the barrier, or at different distances upstream of an obstruction across flowing water.

These findings are illustrated by the following examples :

Uniform Surface Films.

Case 1.

The site was a large pool (area, 25 sq. yds. ; depth, 1 to 3 ft.) surrounded by grasses but open to the sky. The water was clear, and brown algae could be seen at the bottom. The surface was studded with water-lilies, but there was no scum in the spaces between the leaves. The film pressure was measured at 24 places round the periphery of this pool :—

Film pressure (dynes/cm.)	Number of observations
0–15	0
15–20	3
20–25	18
25–30	3
>30	0

Case 2.

The site was a freshwater Palm-Swamp about 3 to 5 acres in extent. The water was muddy and about two feet deep, with a tangle of weeds and rotting palm foliage at the bottom. The water surface, however, seemed clean. The film pressure was measured at 19 places :—

Film pressure (dynes/cm.)	Number of observations
0–5	0
5–10	1
10–15	15
15–20	1
20–25	2
>25	0

Non-Uniform Surface Films.

Case 1.

The site was a forest stream (6 to 9 ft. wide ; 6 to 18 in. deep) which flowed s'owly over a bed of soft mud. At one place some large bamboo canes had fallen athwart the stream causing a blockage. There was a large pool covered with gelatinous bubbly scum on the upstream side of this barrier, and the water escaped underneath. But further upstream the water was crystal clear and its surface clean. The film pressure was measured, in mid-stream, at various distances upstream of the dam :—

Distance upstream from barrier, in feet	Film pressure (dynes/cm.)
0	>45
2	>45
4	>45
6	35–40
8	20–25
10	15–20
12	10–15
14	5–10
20	5–10

Case 2.

The site was an inland *Avicennia* swamp from which water seeped into a nearby river. This swamp had a mud floor, and the depth of water varied from three inches to several feet. The water surface was a patchwork, being penetrated by mangrove roots in the shallower parts and divided up by masses of floating débris where the water was deep. The film pressure was measured at 45 places reached by wading through the swamp :—

Film pressure (dynes/cm.)	Number of observations
0–5	0
5–10	6
10–15	15
15–20	6
20–25	6
25–30	8
30–35	2
35–40	0
>45	2

In all, about 50 sites were visited and the survey yielded a total of 323 determinations of film pressure. An analysis of the results is presented in Table III.

TABLE III.

Observations of the film pressure on natural water surfaces made in a part of the Gold Coast in September to November 1944.

Film pressure (dynes/cm.)	Number of cases observed	Number of cases as percentage of total
0–5	0	0
5–10	62	19·2
10–15	67	20·7
15–20	23	7·1
20–25	43	13·3
25–30	59	18·3
30–35	11	3·4
35–40	5	1·6
40–45	9	2·8
>45	44	13·6

Discussion.

Two interesting points about the natural films studied in this investigation are (1), that the films were of two kinds, which we have distinguished as Uniform and Non-Uniform, and (2), that very large values of the film pressure were quite often found, usually where the water surface was badly fouled with scum. Generally, therefore, it will seldom be possible to obtain a larvicidal oil which is capable of spreading everywhere on the waters in a particular district, and so it is most important that the spreading pressure of the oil which is used shall be the optimum in relation to the surface conditions prevailing at the time of application.

In this connection the broad implications of the data contained in Table III can readily be understood if we recall the definition of film pressure given earlier in this paper. Thus, when we state that the film pressure at a certain place on a water surface was 25–30 dynes/cm. we mean that an oil having a spreading pressure equal to 30 dynes/cm. had been observed to spread at that place but that another oil, with spreading pressure equal to 25 dynes/cm., did not spread. With this in mind we can alter the presentation of the results so as to indicate the spreadability of (larvicidal) oils with different spreading pressures at those places where the tests of film resistance were made. This has been done in fig. 1 (*Note.*—The "normal" curve shown in this diagram has been fitted by drawing the best straight line through a plot of the experimental values on logarithmic probability graph paper.) It is at once obvious that whereas the spreadability of an oil would have increased rapidly as its spreading pressure was raised from 5 to 30 dynes/cm., approximately, the improvement to be expected from a further substantial increase of spreading pressure would have been relatively small (as well as being much more difficult, and costly, to achieve). It is considered, therefore, that the optimum spreading pressure for a larvicidal oil intended for distribution in the district specified at the time when this survey was made would have been about 30 dynes/cm.

In conclusion, it is of interest to record that, while making this investigation, the writer was able to observe what happened when larvicidal oils having spreading pressures between 25 and 30 dynes/cm. were deposited in the form of fine spray on the water at some of the sites visited. Generally, the spreadability of these oils seemed to be adequate, and it was not uncommon for a continuous film to be formed where only a light deposit of droplets had fallen.
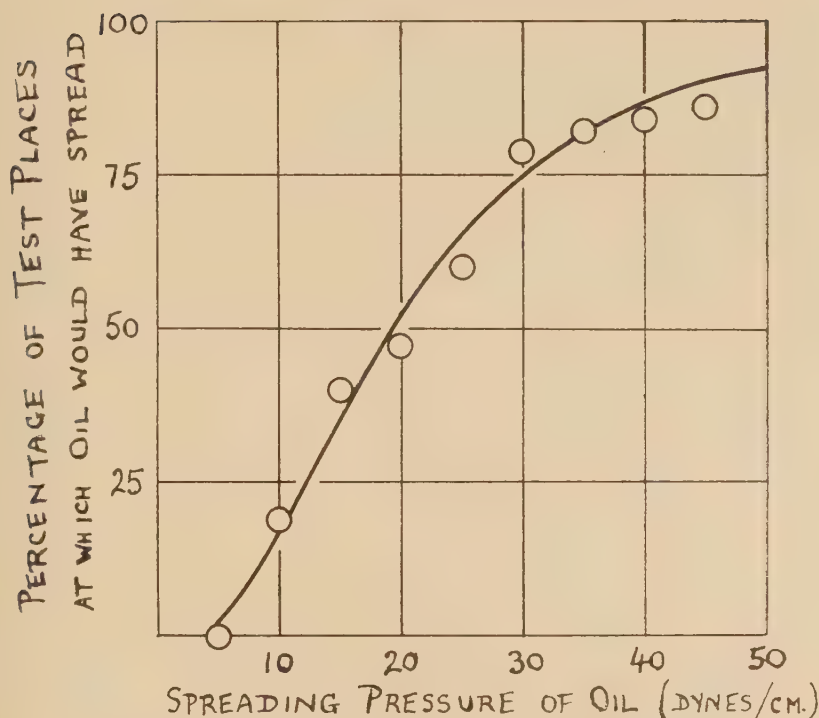
Fig. 1.—Spreadability of oils as a function of spreading pressure.

Summary.

The spreading of larvicidal oils is hindered by the presence of natural films upon the surface of standing water. The film pressure, which measures the resistance offered to the spreading of oil, can be determined in the field by a simple method depending on the use of standard spreading liquids.

It seemed reasonable to suppose that by making tests on the waters at many different places it should be possible to estimate the optimum spreading properties required in a larvicidal oil *before* its distribution began in a particular district. An attempt to do this was made in a part of the Gold Coast in 1944 and the investigation showed that in that region, and at that time, the spreadability of an oil with spreading pressure equal to 30 dynes/cm., approximately, would have been adequate for larvicidal purposes. It was also found that the natural films, which could be either visible or invisible, were of two physical types distinguishable as " uniform " and " non-uniform " according to the observed variation of the film pressure at a single site.

Acknowledgements.

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TSETSE FLIES CARRIED BY RAILWAY TRAINS IN KENYA COLONY.

By E. Aneurin Lewis.

Chief Field Zoologist, Kabete, Kenya Colony.

Following the construction, some 50 years ago, of the main railway from Mombasa on the east coast to Nairobi 330 miles inland, it has been common experience to find tsetse flies entering carriages of passenger trains at stations situated in tsetse-infested areas traversed by this railway (Ross, 1911 ; Austen, 1911 ; Neave, 1912 ; Newstead & others, 1924 ; Lewis, 1942a & b, 1947). It was thought (Milne, 1910) that flies were attracted to the lights when trains waited during the night at certain stations, and were carried in the compartments for distances of 150 miles or more. Attention was drawn, in the first decade of the present century, to the possible serious consequences of thus spreading tsetse flies and increasing the incidence of human sleeping sickness and animal trypanosomiasis in Kenya Colony, and in other territories similarly affected (Report of Sleeping Sickness Conference held at the Foreign Office, London, January 19, 1911—summary in Bull. Sleep. Sickn. Bur., **3,** pp. 146–149), and it was suggested that investigations should be instituted to determine the distribution of tsetse flies along the line of the railway, and to recommend measures of control.

Fear of spreading human sleeping sickness by flies carried by trains between Mombasa and Nairobi seemed to have been dispelled when it was realised that the fly belt through which the trains travelled contained species of tsetses (*Glossina longipennis*, Corti, *G. pallidipes*, Aust., and *G. brevipalpis*, Newst.) not usually incriminated in the transmission of the human disease. Animal trypanosomiasis in the country between the coast and the highlands had not assumed the importance attached to it in recent years except, perhaps, in so far as it affected the safety of transport of imported stock. In a " Report on the Progress of the Mombasa-Victoria (Uganda) Railway, 1897–1898," published in 1901, His Majesty's High Commissioner in the East Africa Protectorate stated that " the country in parts being infested with tsetse fly as far as mile 220 (a little beyond Kiboko), the mortality amongst transport animals would have been very heavy had larger transport arrangements been made for expediting works far ahead of the rails, and an enormous outlay would have been entailed which circumstances hardly seem to justify." It was also claimed, quite reasonably at this very early period, that since the advent of the railway, horses could be brought up from the cost to Ukamba for travelling purposes while formerly it was the custom in that country to make long journeys on foot owing to the impossibility of conveying horses from the coast through the intervening fly belts. Later, trucks for the transport of imported high-grade farm animals to the settled interior highlands of the Colony were provided with wire gauze of small mesh to prevent tsetse flies biting the animals and infecting them with trypanosomes.

These provisions having been made, little further importance seems to have been attached to the part played by trains in the dispersal of tsetse flies, and no case appears to have been presented to indicate its full significance.

In this account of investigations conducted under the auspices of the Kenya Tsetse and Trypanosomiasis Committee, it is shown that trains travelling through fly belts carry large numbers of tsetses into occupied fly-free areas ; that goods trains, perhaps because of their greater frequency, convey more flies than passenger trains. Outbreaks of animal trypanosomiasis which can be explained only as a result of this form of tsetse dispersal have occurred in the past, and still occur, among high-grade dairy stock and in herds of native cattle both at the coastal end of the

railway and between the inland limit of the fly belt and the Nairobi district. Losses from the disease arouse a feeling of insecurity and a reluctance to utilise land adjacent to the railway to its full potential capacity. The continuous introduction of flies on to wide strips of the land immediately outside the permanent fly belt will almost certainly be an obstacle to the successful settlement of African or other stock farmers along what is otherwise a useful line of communication.

It is submitted that the situation calls for an early attempt to displace the flies from all trains at least as soon as practicable after the trains have left the permanent fly belt.

A Fly Survey along the Rail Route.

The rough limits of the fly belt through which the railway passes from the Kenya coast to the central Highlands have been known for a large number of years, but the precise limits and the nature of the infestation have not been determined until recently. In the course of a detailed survey of the tsetse distribution in the Colony, special attention was paid to the country on both sides of the Mombasa-Nairobi railway and of the main road which runs close to and parallel with it.

The infestation in the neighbourhood of the railway may appropriately be divided into the following five sectors :—

(*a*) The Coastal sector which extends from Mombasa to near Maji ya Chumvi station—a distance of about 33 miles.

(*b*) The Samburu Marginal sector stretching from near Maji ya Chumvi to about 2 miles up-line from Samburu station—about 10 miles in all.

(*c*) The Main fly belt from near Samburu to Kiboko station next to Makindu—a stretch of approximately 177 miles.

(*d*) The Simba Marginal sector extending from about the Greater Kiboko river for 8 or 9 miles to Simba.

(*e*) The Lower Highlands sector from Simba to Nairobi—a distance of about 101 miles.

The country adjacent to the railway in the coastal sector is not uniformly or heavily infested with tsetses. Up-country trains from Mombasa travel through plantations of coconut palm interspersed with mango trees, cashew and citrus in the Changamwe-Miritini peninsula. Patches of dense, shrubby undergrowth assist in providing conditions favourable to light dispersal of *G. pallidipes* from the permanent, littoral infestation (Ngu Tatu—Shanzu) north of Mombasa island. The Mwachi forest, south of the railway near Mazeras, harbours *G. austeni*, Newst., which, however, is not readily—if at all—attracted from its haunts in the dense forest vegetation. *G. pallidipes* exists with *G. austeni* on the Rabai hill and small numbers of the former sometimes range beyond the forested hill to the scrubland which stretches towards Mazeras and the railway. This fly, and perhaps other species also, rarely appears on trains unless the fly population near the railway is higher than it is in this locality. *G. brevipalpis* has, on occasions, been collected in this sector, but no specimens have yet been taken from trains or near the railway. *G. longipennis*, on the other hand, is found on down-trains and in localities adjoining the railway and this is a species which does not naturally exist in the coastal zone. Its haunts are farther inland. Yet, specimens have often been caught at or near Miritini, Mazeras and Mombasa, and a few have been collected on Flora Point, immediately south of Mombasa island and in the coconut plantations near Changamwe. These, it will be shown later, could only have been brought into the area, chiefly on trains from up-country. It is not unusual to obtain one or more *G. longipennis* in the vicinity, or among herds, of cattle in the open fly-free grazing belt (Ndavaya-Mariakani-Ganzi) which crosses the railway from near Mariakani to

Maji ya Chumvi, and a few have been found feeding on cattle at the Mariakani Veterinary Centre, not far from the station.   Foci of *G. pallidipes* and *G. austeni* exist at Kigutu and Mlungani south of the railway and below Maji ya Chumvi but the flies are not, apparently, attracted to passing trains.

The vegetation in this sector, consisting chiefly of scattered plantations, some-what small isolated forests, and numerous plots of cultivation, low scrub or grazing, tends to discourage heavy widespread fly infestation.   The African inhabitants keep either a few cattle, sheep and goats or, in the fly-free Ndavaya-Mariakani-Ganzi belt, numerous large herds of cattle.   Trypanosomiasis is not uncommon and although it does not seem to perturb the African owners, it has proved to be a con-siderable obstacle to European and Indian stock-owners who have attempted to develop farmland and establish dairy farming from near Mombasa to Miritini or Mazeras.   The attempts have not met with success.   Stock-farming has been abandoned, farms have frequently changed hands or have been left in a more or less derelict state.   It is in this sector that operations are in progress to reclaim an area near Miritini, for the transfer of stall-fed, high-grade dairy cattle from within the township of Mombasa.   Reclamation on a larger scale in conjunction with the agricultural development of the area is contemplated ; its success will depend, to a significant degree, on the efficacy of measures to prevent the introduction of tsetse flies from the heavily infested fly belt further inland.

Soon after passing Maji ya Chumvi, up-trains enter more thicketed and drier country.   *G. pallidipes* is fairly common especially in the denser vegetation along the usually dry river courses.   The fly is also lightly scattered throughout the sector and, except for an occasional small focus of *G. brevipalpis*, is the only species so far found to exist up to Mackinnon Road.   *G. longipennis* occurs near the railway, and sometimes in the bush a little distance away.   No evidence has yet been found of its breeding in the area.   Its numbers tend to increase slightly west of Samburu.

The area is occupied up to about 2 miles beyond Samburu.   The African inhabitants cultivate some of the land, and they keep a number of cattle and goats on rather small areas of grassland which break up the continuity of the thicketed bush.   The cattle population falls rapidly towards Samburu, due, in part at least, to the increase of fly and the incidence of trypanosomiasis.   The prospects of improvement in the stock situation by bush-clearing to eliminate *G. pallidipes* in this occupied marginal sector are clouded over by the knowledge that *G. longipennis* appears to be continually brought into the area, and if the amount of traffic increases, with the establishment of a military base in and around Mackinnon Road, the " importation " of fly may be on a larger scale.

The main belt which is to all practical purposes uninhabited except in the neighbourhood of the townships at or near railway stations comprises four species of tsetses, namely, *G. longipennis*, *G. pallidipes*, *G. brevipalpis* and *G. austeni*.

G. austeni occurs in one locality only—on the Voi river at the foot of the Voi hills between one and two miles east of the railway line.   *G. longipennis* which, for the first time in this, or the coastal sector, appears within its natural, unaided range of dispersal at about 6 or 7 miles north-west of Mackinnon Road and about 65 miles from Mombasa.   Its density in the immediate vicinity of the railway is not particularly high, though large numbers have, on occasions, been taken by fly-boys. Breeding-places have been located a mile or more below the railway, more particularly south of Buchuma, Maungu and Ndara stations.   Flies on the wing ranging from these breeding centres towards and over the railway are, as will be seen later in this paper, augmented by others brought in by trains and sometimes, no doubt, by constant road traffic.   *G. pallidipes* is also widely distributed in the intensively—and massively—thicketed stretch between Mackinnon Road and Voi.   Many open glades provide suitable hunting grounds for this species, while the deciduous shrubs and the dryness of the climate help to keep the infestation at a relatively low level.
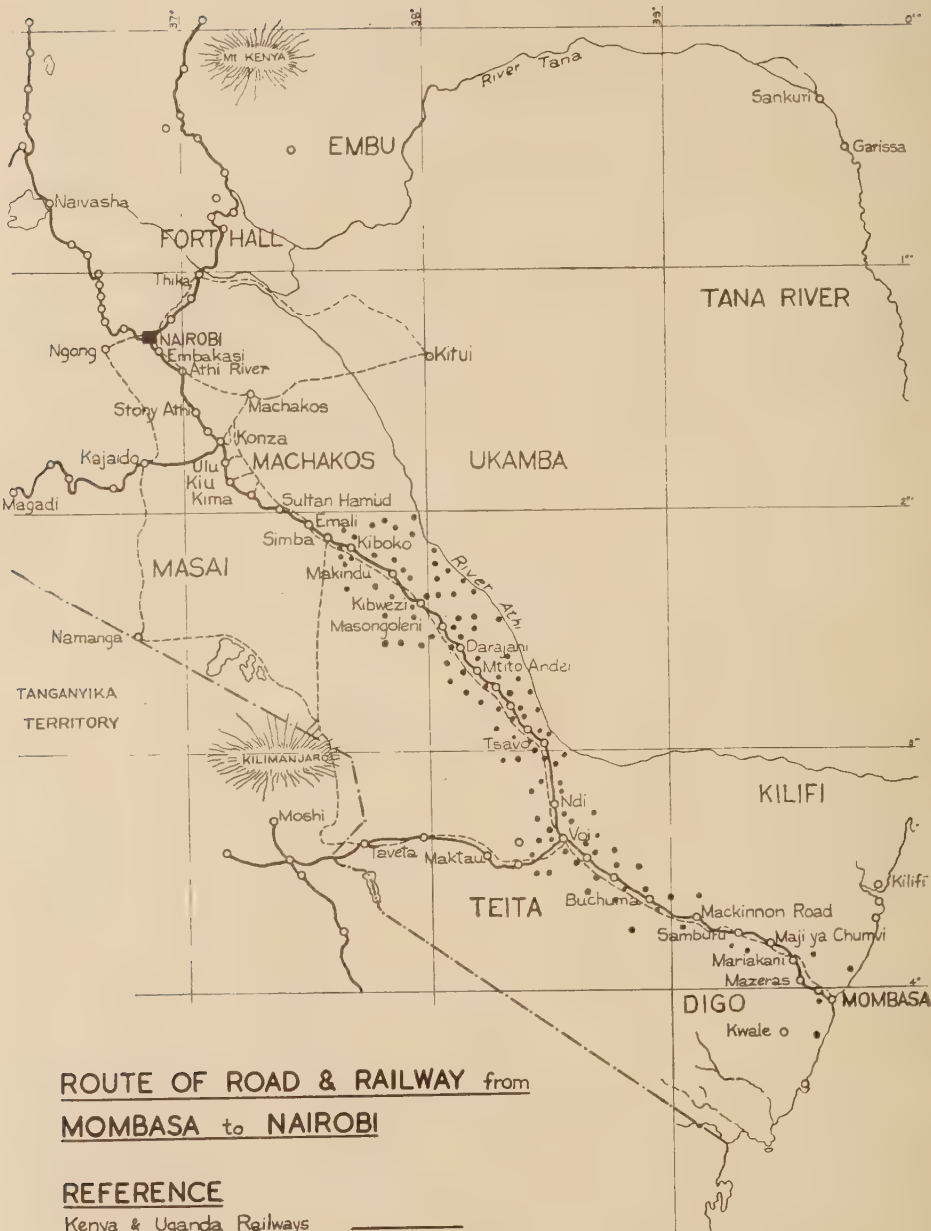
ROUTE OF ROAD & RAILWAY from
MOMBASA to NAIROBI

REFERENCE
Kenya & Uganda Railways　————————
Main Roads　　　　　　- - - - - - - -

Scale of Miles　20 10 0　　20　　40　　60　　80　　100

MAP I.　The natural distribution of tsetses near the road
　　　　 and railway is indicated by dots.

Many thousands of acres around Voi have been cleared of bush and planted with sisal. They are themselves free of fly ; but strips of uncleared bush and a few neglected overgrown plantations harbour both *G. longipennis* and *G. pallidipes*. Other small areas of treeless grassland and patches of savannah contribute to conditions which are not favourable to dense infestation by either of these species. Herds of cattle from the Teita, Mbololo and Sagalla hills are often pastured in these localities infested by *G. longipennis*, and they suffer from trypanosomiasis which is often treated by the district veterinary officers.

Between Voi and Tsavo stations, a stretch of about 30 miles, *G. longipennis* increases in numbers. A survey of the country alongside the railway failed to reveal widespread breeding sites, but puparial shells of *G. longipennis* have, in the past, been found under tree-trunks and logs near the main road. The absence of breeding-places, except close to the Tsavo river, is probably due to the scarcity of shade trees over the low thorn scrub, and to the heat and dry conditions which prevail for many months of the year. Flies on the wing are most abundant along the railway track where their activity is a subject of frequent comment by railway employees and others acquainted with this section of the country.

The infestation—by *G. longipennis* alone—continues and becomes more intensified from Tsavo to near Mtito Andei where *G. pallidipes* again makes its appearance. The flies are very abundant in this section, and breeding-places are widespread and numerous. The vegetation, generally, consists of dense, tall thicket as undergrowth to a close formation of canopy trees. Open glades and stretches of orchard-like country interrupt the continuity of the dense forest, but provide suitable feeding grounds for ranging flies. This part of the country through which the railway runs may be considered as the core of the inland fly belt as far as *G. longipennis* is concerned. The density of the fly population reported by the tsetse survey unit is supported by previous observations and by the experience of railwaymen who have noted that trains attract the largest numbers of flies usually between Ndi and Mtito Andei stations.

G. longipennis persists beyond Mtito Andei, but its breeding-places diminish ; they are few and far between. Whether this is due to the change in vegetation only or to a combination of this and other factors is not known ; but it may be stated, at this stage, that the altitude increases gradually from sea-level at Mombasa to 1,180 ft. at Mackinnon Road, 1,830 ft. at Voi, 1,530 ft. at Tsavo, 2,000 ft. at Mtito Andei and 2,986 ft. at Kibwezi.

From near Mtito Andei through Darajani and Masongaleni stations to Kibwezi, the composition of the fly infestation changes rapidly. *G. longipennis* is still common and widely distributed but *G. pallidipes* is now the predominating species. Beyond Masongaleni, *G. longipennis*, as an inhabitant species, tends to disappear. *G. brevipalpis* makes its appearance and occurs abundantly in a few foci situated in the densely wooded river-beds. It is likely that some of the specimens of *G. pallidipes* and *G. brevipalpis* collected from trains further up-line are brought from this locality.

G. pallidipes ranges widely over the land immediately south and west of the railway for at least 5 miles beyond Kibwezi. It occurs, to a less degree, up to Makindu, and through the more wooded savannah from Makindu to the Kiboko river. On the northern and eastern side of the railway from Kibwezi a number of relatively fly-free enclaves surrounded by bands of infested territory seem to have arisen either as a result of penetration and settlement by native families or because the natural vegetation has been, and is, unfavourable to fly. It is in this locality, east of the railway, that Government has recently acquired land for improved African settlements and agricultural development. Successful settlement and subsequent development will depend to some extent on the effective removal of flies from trains passing through the area to be reclaimed by other measures.

G. longipennis, still present all along the railway and in somewhat isolated patches away from the railway line, appears in gradually increasing numbers from Makindu to the Kiboko river. The density of this species is high in the Kiboko riverine vegetation where *G. pallidipes* and *G. brevipalpis* also are not uncommon. Breeding-places of all three species are common.

Attempts to develop at least parts of this area by Indian settlers have failed, apparently on account of losses amongst cattle introduced for cultivating the land. A few cattle graze over a small cleared piece of land near Makindu station, and fruit and vegetables are grown on another close to the Lesser Kiboko river. Otherwise, this stretch of about 8 miles between Makindu and Kiboko is unoccupied.

The Greater Kiboko river, approximately one mile north of the Lesser Kiboko, marks the inland border of the main fly belt. Neither *G. pallidipes* nor *G. brevipalpis* disperses far into the country north of its riverine forest. *G. longipennis*, however, spreads into more open country for about 2 miles or more, and it finds suitable places for breeding under fallen logs scattered over a narrow strip of open woodland. Lighter infestations of *G. longipennis*, *G. pallidipes* and *G. brevipalpis* exist on parts of the Simba and Muoni rivers which run fairly close to, and on the north of, the railway between Kiboko and Simba stations and beyond. The very open nature of the country generally, however, provides an effective barrier to widespread infestation and restricts the natural range of flies to the linear riverine vegetation and patches of heavily thicketed country. It is unlikely, therefore, that many tsetses from these rivers are attracted to trains travelling between Simba and Kiboko. On the other hand, the presence of flies near the railway and in the upper reaches of the Simba river—close to the railway—indicates that flies brought from the main fly belt by trains are frequently shed along the route and thus aided in their dispersal into country that would otherwise be inaccessible.

It can confidently be stated that the open country beyond the Simba marginal sector of infestation is itself free from a natural fly infestation and serves as an effective barrier to unaided advance of the fly belt. The whole distance from Simba to Emali (about 14 miles) is practically treeless plain—extensions of the vast plains of the Masai reserve south of the railway line. The altitude at Simba is 3,347 ft., at Emali 3,792 ft., at Sultan Hamud 4,025 ft., and at Nairobi, the farthest point in Lower Highlands according to the classification of sectors adopted in this report the altitude is 5,453 ft.

There is, nevertheless, what was at one time considered to be a focus of permanent infestation in the forested foothills of the Olmundus range which stretches (immediately west of the railway) from Emali station to Sultan Hamud (Lewis, 1942a). Repeated surveys in the area have modified the view previously held. They have indicated an instability in the fly population and disparity of breeding-sites which suggest that the flies—*G. longipennis*, *G. pallidipes* and *G. brevipalpis*—are periodically re-inforced by others brought into the locality. A similar state of affairs exists on the very much less wooded occupied European farm on the opposite side of the railway between Emali station and Sultan Hamud, and on both sides of the railway line as far at least as Ulu station (5,253 ft.) about 58 miles from the edge of the main fly belt at the Greater Kiboko river. Pupae or puparial shells of the different species have on a few occasions been found at Olmundus. Their location and distribution point to accidental or fortuitous rather than selected siting for deposition of pupae. In other places where flies, usually *G. longipennis*, are commonly found breeding-places have not been discovered in the course of extensive and prolonged searches. *G. brevipalpis* and *G. pallidipes* are very rarely collected in the Lower Highlands sector outside the Olmundus focus. The few specimens obtained, from time to time, over many years have been caught in trains. The survey unit has not been able to find either of these two species on farms or on the plains between Sultan Hamid and Nairobi. *G. longipennis* on the other hand, is not uncommonly encountered.

For instance, the number of flies collected by natives on the Emali-Sultan Hamud farm (No. 7390) occupied by Mr. Thompson, and submitted weekly or fortnightly for identification, amount to about 250 to 500 annually. *G. longipennis* always by far the dominant, if not the only, species in this sector, is scattered widely—though not abundant—in the more thickly bushed locality in the angle formed by the railway and the main road to a distance of approximately 4 miles from Sultan Hamud. A smaller number of flies was found farther up the line and they were collected in widely separated spots; sometimes in wooded drainage lines, sometimes in open country and, except on very rare occasions, practically always within a mile or two of the railway. Single specimens have been obtained from most unusual localities which, however, are within a short distance from the railway, namely, in Nairobi and its suburbs, Ruaraka, Naivasha; and, recently, a specimen found harassing horses, on a farm at about 7,200 ft. near Limuru station.

The Simba marginal area, defined earlier in this paper, is at least seasonally utilised by the Masai and the Ukamba natives for grazing large herds of cattle ; and although trypanosomiasis is known to affect some of the herds, the extent of infection seems not to have been ascertained. Blood smears from many sick animals have shown trypanosomes, and information from natives indicate periodic losses from the disease. Trypanosomiasis is constantly present on Mr. Thompson's farm at Emali and although its incidence varies, the mortality is often considerable in spite of diligent therapeutic treatment. The history of other occupied farms, most of which carry valuable high-grade or pure-bred dairy and dual purpose cattle, contains evidence of cases and outbreaks of trypanosomiasis which have caused serious losses or aroused a fear of losses adversely affecting stock or dairy farming for which these Lower Highlands seem so eminently suitable and conveniently situated.

Tsetse Flies on Road Vehicles and on Trains.

The earliest record of tsetse flies in carriages on the Kenya and Uganda railway appears to be that of *G. pallidipes* collected in 1903 by Dr. Moffat at Kibwezi and Simba (Austen, 1911). Subsequent records, which include *G. longipennis*, cover the years up to 1948. It is evident, therefore, that trains on the Mombasa-Nairobi line have been carrying flies for at least 44 years to date. The potential danger of this form of tsetse dispersal was appreciated and published as early as 1910 when it was stated at a Conference of Medical and Veterinary Officers held in Nairobi in April of that year that " The risk of conveyance of tsetse by the Uganda Railway is diminished by the fact that these species (? *fusca* (*brevipalpis*), *longipennis* and *pallidipes*) do not exist at the high altitudes ; that such flies may be carried 125 miles shows that special measures will have to be taken as the tropical African railways increase in number and mileage." It was also recommended that the carriage of tsetse flies in trains was a matter which should engage attention.

A full investigation of the problem was deferred partly, apparently, for reasons already stated in the introduction to this paper, and partly also to the lack of trained staff to carry out the work.

On the formation of a Tsetse and Trypanosomiasis establishment in the Colony, the rôle of road and railway traffic as agents of tsetse fly dispersal was more closely studied than hitherto.

Motor cars, lorries and military convoys utilising the main Mombasa-Nairobi road during World War II were examined about the latter half of 1945, at Emali ; up-trains stopping at this station were also searched for flies. The initial steps taken were exploratory. From 1,084 cars, only one fly (*G. longipennis*) was taken during a period of 7 months, whereas 145 (*G. longipennis* and *G. pallidipes*) were collected from 1,809 lorries in the same period. The largest number of flies was found on lorries travelling in convoy. They varied from 4 to 33 flies for convoys

of 10 to 64 vehicles. The most favourable places on the vehicles were under the tarpaulin cover of the van which was usually open at the back ; it seemed as if the draught created by the moving lorries swept the flies in through the opening at the rear. A few flies were found under the chassis and, less frequently, on the sides of the vehicles when stationary. On a few occasions, single specimens of *G. longipennis* were collected, near Emali, from lorries which had not been within 18 miles of the main fly belt, and it is assumed that these flies had come from other vehicles which had passed through the fly belt, or from trains. Single specimens were also taken on each of seven occasions from lorries travelling separately.

The number of tsetses collected from road vehicles dropped so considerably on the cessation of military convoys that the de-flying of road traffic was discontinued. No doubt small numbers are being carried beyond Emali, and it seems likely that flies which have reached the wooded farms near Sultan Hamud by train are carried still farther along the road by cars and lorries travelling inland from the railway and towards Machakos and Nairobi.

A preliminary search of up-trains in 1945 produced much higher catches of tsetses than cars and lorries—much higher, in fact, than had been expected.

Passenger trains were examined by two trained African assistants who boarded the train at Emali station and, sometimes accompanied by a European officer, searched the corridors and toilet compartments of the first and second class coaches, and throughout the third class carriages. They de-trained at Sultan Hamud station, approximately 9 miles from Emali.

The catches from mid-May to mid-December, 1945, amounted to 2,711 flies from 241 passenger trains ; they comprised 2,612 *G. longipennis*, 79 *G: pallidipes* and 20 *G. brevipalpis*.

Normally, only one passenger train travels daily from Mombasa to Nairobi. It arrives at Emali in the dark hours of the morning (about 4.30 a.m.) so that the outside of the coaches cannot readily be examined. (Searches conducted in daylight farther up the line, e.g. Athi River station, have resulted in catches of up to 10 flies per train on various external structures of passenger coaches.)

Goods trains are more frequent. Only those travelling by day were subjected to de-flying. Many of these did not stop at Emali. Others stopped for a minute or two, not long enough to enable the fly-boys to catch more than a relatively small number of the flies seen.

The total catch from 248 goods trains which stopped, whatever the period, at Emali was 1,291 tsetses comprising 1,226 *G. longipennis*, 44 *G. pallidipes* and 21 *G. brevipalpis*. An additional 214 goods trains passed, non-stop, through Emali station, and it was reasonable to assume that they carried flies beyond this station. This assumption was checked, to some extent, by boarding a few trains at Emali. One or more European officers kept a number of flies on the trucks under observation as the train was moving. At Sultan Hamud, during a longer wait, the same trains were examined again. The catches from eleven goods trains first at Emali and again at Sultan Hamud are given in Table I. They show that flies are carried at least as far as the latter station in the Lower Highlands sector of the railway line.

In 1946, it was possible to carry out a more systematic study of the significance of the dispersal of tsetse by trains. By arrangement with the authorities of the Kenya and Uganda railways, de-flying of trains was carried out at both Emali and Sultan Hamud. A team consisting of five African assistants in charge of a senior African assistant specially instructed in de-flying by hand (using a fly-swatter) was posted at each station. They were frequently visited by Mr. Thompson, Emali, whose help in several other directions also is gratefully acknowledged, and periodically by a Tsetse Officer. The catches were checked weekly, analysed, compared with the record sheets, and destroyed.

TABLE I.

Station	G. longipennis			G. pallidipes			G. brevipalpis			No. of trains
	Total	M.	F.	Total	M.	F.	Total	M.	F.	
Emali	138	79	59	2	1	1	7	5	2	11
Sultan Hamud	105	60	45	3	1	2	2	2	0	11
Totals	243	139	104	5	2	3	9	7	2	11

M=males ; F=females.

The total number of flies, comprising *G. longipennis*, *G. pallidipes* and *G. brevipalpis*, thus collected over a period of 52 weeks (from July, 1946, to June, 1947, inclusive) was 24,540—an average of nearly 472 flies per week. The total for 77 weeks (from July, 1946, to December, 1947, inclusive) was 37,982—an average of 493 flies per week. The minimum number recorded for a week was 202, and the maximum 1,229 flies.

These totals include 5,671 tsetses which were taken from the inside of passenger trains. Those collected in the first, second and third class carriages and in the toilet compartments amounted to 1,765, 1,622, 1,846 and 438 respectively. Among them were 228 females containing visible pupae which were about ready to be deposited. In fact, many of them did pass viable pupae after transfer to glass containers.

Train compartments occupied by passengers were not examined, but it is known from personal experience and from other reports* that specimens of tsetses enter compartments either directly through open windows or indirectly by way of corridors. These are usually caught and destroyed by passengers and, sometimes, sent in for identification. Flies are not infrequently seen at night fluttering on the window-panes of lighted compartments or against the gauze panels provided by the railway company to exclude mosquitos and other insects. It may be reasonable to infer in the circumstances that the tsetses are attracted by the lights. That light is not the primary attraction is demonstrated by catches from goods trains which are known to have passed at night, without lights, through the main fly belt.

The total number of tsetses collected from goods trains amounted to 20,709 and 32,311 in 52 and 77 weeks respectively ; and the weekly averages, in round figures, to 398 and 410 flies, considerably greater than the total and average per week (74 flies) from passenger trains.

The catches are analysed further in Tables III and IV and may be compared with those given in Table II which summarises the catche from passenger trains. They show that very many more tsetse flies are brought into the Lower Highlands than was indicated by previous records, and that the introduction of flies is continuous. The range of numbers per train varies considerably. From about 30 to 80 were commonly collected from one goods train at Sultan Hamud, and while the average from 1,280 trains was between 18 and 19, the maximum reached 227 flies. Only on 3 trains examined at this station were no flies found. The smaller total catch at Emali is attributable to the smaller number of trains (827) examined, while the lower average (9 to 10 flies) per train is due to the fact that many of these trains stop at the station only for about a minute or two and the fly boys are often unable to collect more than a small proportion, if any, of the flies seen. Moreover,

*One passenger recently collected 15 *G. longipennis* which had entered a compartment by the open window.— E.A.L.

an additional 492 goods trains did not stop at Emali and must have taken numerous flies farther up the line ; nor were trains which arrived at Emali and Sultan Hamud after nightfall, or before break of day, examined by the de-flying teams.

TABLE II.

Tsetse collected in passenger trains between Emali and Sultan Hamud.

Month	G. longipennis			G. pallidipes			G. brevipalpis			Total No. of Flies	No. of trains exam'd	Average No. flies per train
	M.	F.	Total	M.	F.	Total	M.	F.	Total			
1946												
July ...	127	126	253					3	3	256	31	8·26
Aug. ...	144	165	309		1	1	1		1	311	31	10·03
Sept. ...	107	109	216				1		1	217	30	7·23
Oct. ...	163	119	282	2	1	3			-	285	31	9·19
Nov. ...	161	101	262	6	4	10	1		1	273	30	9·10
Dec. ...	158	116	274	5	7	12	1	2	3	289	31	9·32
1947												
Jan. ...	246	164	410	13	8	21	3	4	7	438	31	14·13
Feb. ...	244	171	415	10	11	21	1	1	2	438	28	15·64
Mar. ...	204	144	348	1	9	10	2	1	3	361	31	11·64
Apr. ...	292	122	414	1		1	1		1	416	30	13·86
May ...	202	76	278							278	31	8·97
June ...	154	110	264	2	1	3		2	2	269	30	8·96
Totals ... (52 weeks)	2202	1523	3725	40	42	82	11	13	24	3831	365	10·50
July ...	161	138	299	4	2	6		2	2	307	31	9·90
Aug. ...	110	69	179	1	3	4				183	31	5·90
Sept. ...	122	114	236	4	3	7	1		1	244	30	8·13
Oct. ...	192	172	364	5	3	8	1	1	2	374	31	12·09
Nov. ...	274	248	522	2	2	4	1	1	2	528	30	17·51
Dec. ...	122	77	199	4	1	5				204	31	6·58
Totals ... (77 weeks)	3183	2341	5524	60	56	116	14	17	31	5671	549	10·33

M. = males ;   F. = females.

In the light of this evidence, and the findings of the survey unit, no doubt can be entertained that trains which have passed through the main fly belt carry large numbers of flies many of which escape *en route*. De-flying by hand is admittedly not entirely satisfactory, and although it has brought about a considerable reduction in the numbers of tsetses carried up-country beyond Sultan Hamud, it has had no effect on trains passing these points at night. Many have escaped capture even in the day-time and have been conveyed on the trains proceeding up the line towards Nairobi. This was demonstrated by catches made at other stations between Sultan Hamud and Nairobi. The de-flying team, in this case, comprised 16 Africans under the constant supervision of a European officer. It operated at each intermediate station for 3 days when all trains stopping at the station, during the day, were examined. The results are shown in Table V.

These flies were collected from trains which had already been '' de-flied '' at Emali or Sultan Hamud, or both. If the figures represent the state of affairs throughout the year when 823 goods and 365 passenger trains (see totals in Tables IV and II respectively) travel from Mombasa, it is estimated that no less than 7,873 flies are carried, annually, on up-trains which pass by day beyond Sultan Hamud station.

No flies have yet been found on down-trains in the Lower Highlands (i.e. Nairobi-Simba) sector of the railway. Passenger trains to the Coast pass through the Sultan Hamud to Makindu section during the hours of darkness and goods trains seldom stopped at the intermediate stations sufficiently long to enable the itinerant de-flying

team to make a thorough search. After passing the inland border of the main fly belt, the goods trains had gathered—on their arrival at Makindu—41 flies, and the passenger trains four flies during the three-day de-flying period. The average number per train remained at about the same level in the case of the goods, but higher on the passenger trains beyond Kibwezi. By the time the former, more especially, had reached Tsavo, the total number of flies and the average per train were high. A drop was recorded at Voi, and again at Mackinnon Road, after which relatively small numbers were taken at Samburu and Mariakani. Previous inspections from Mariakani to Mombasa gave results somewhat similar to the last two stations. Table VI shows the catches from both passenger and goods trains from Makindu to Mariakani, whereas Table VII shows catches from up-trains at the same stations, on the same days and under the same conditions of itinerant de-flying. When the average figures in both Tables relating to goods trains are plotted as in fig. I, a significant correlation is revealed in so far as the trend of the curves is concerned.

TABLE III.

Tsetses collected from goods trains at Emali.

Month	G. longipennis			G. pallidipes			G. brevipalpis			Total No. of Flies	No. of trains exam'd	Average No. flies per train
	M.	F.	Total	M.	F.	Total	M.	F.	Total			
1946												
July ...	170	140	310					2	2	312	41	7·60
Aug. ...	158	130	288		1	1				289	45	6·42
Sept. ...	132	95	227							227	42	5·40
Oct. ...	146	90	236	3	1	4				240	30	8·00
Nov. ...	189	102	291	9	2	11	8	5	13	315	37	8·51
Dec. ...	226	155	381	5	5	10	2	1	3	394	42	9·38
1947												
Jan. ...	197	140	337	4	1	5	2	1	3	345	33	10·45
Feb. ...	219	110	329	2	5	7	3		3	339	36	9·42
Mar. ...	309	192	501	7	4	11		1	1	513	58	8·84
Apr. ...	488	159	647	1	2	3		1	1	651	55	12·74
May ...	344	153	497	4	2	6		2	2	505	52	9·71
June ...	464	337	801	6	5	11	3	7	10	822	51	16·12
Totals ... (52 weeks)	3042	1803	4845	41	28	69	18	20	38	4952	522	9·49
				Through trains = 327								
July ...	480	305	785	8	6	14	6	6	12	811	47	17·26
Aug. ...	398	343	741	2	2	4		1	1	746	56	13·32
Sept. ...	189	147	336	3	5	8		3	3	347	55	6·31
Oct. ...	230	155	385	3	4	7	1		1	393	47	8·36
Nov. ...	278	202	480	3	5	8	3	1	4	492	48	10·25
Dec. ...	310	172	482	9	3	12		1	1	495	52	9·52
Totals ... (77 weeks)	4927	3127	8054	69	53	122	28	32	60	8236	827	9·96

Through trains = 327 + 165 = 492

The maximum catches are recorded, on both up- and down-trains, within the Kibwezi-Tsavo sections of the railway, on the down-trains between Mtito Andei and Tsavo and on the up-trains between Tsavo and Mtito Andei. This, it will be noted, coincides with the results of the survey unit which marked the neighbourhood immediately down-line of Mtito Andei as " the core of the inland fly belt as far as G. longipennis is concerned ". The numbers of tsetses collected in the course of the itinerant de-flying were :—

Up-trains : 1,887 G. longipennis ; 76 G. pallidipes ; 11 G. brevipalpis.

Down-trains : 1,647 G. longipennis ; 20 G. pallidipes ; 6 G. brevipalpis.

TABLE IV.
Tsetses collected from goods trains at Sultan Hamud.

Month	G. longipennis			G. pallidipes			G. brevipalpis			Total No. of Flies	No. of trains exam'd	Average No. flies per train
	M.	F.	Total	M.	F.	Total	M.	F.	Total			
1946												
July ...	586	463	1049	4	2	6	5	1	6	1061	72	14·73
Aug. ...	476	348	824	4	3	7	2	3	5	836	57	14·66
Sept. ...	411	316	727		1	1	2		2	730	66	11·06
Oct. ...	532	338	870	7	5	12	2	3	5	887	65	13·60
Nov. ...	628	392	1020	27	13	40	11	11	22	1082	63	17·17
Dec. ...	977	610	1587	25	9	34	10	7	17	1638	63	26·00
1947												
Jan. ...	787	505	1292	4	6	10	12	8	20	1322	56	23·68
Feb. ...	481	303	784	9	2	11	8	4	12	807	60	13·45
Mar. ...	944	469	1413	10	8	18	8	3	11	1442	83	17·37
Apr. ...	994	536	1530	6	11	17	9	3	12	1559	83	18·78
May ...	1129	670	1799	21	7	28	8	2	10	1837	78	23·55
June ...	1530	980	2510	25	9	34	5	7	12	2456	77	31·89
Totals ... (52 weeks)	9475	5930	15405	142	76	218	82	52	134	15757	823	18·89
July ...	816	568	1384	15	6	21	8	7	15	1420	67	21·19
Aug. ...	863	763	1626	7	6	13	2	5	7	1646	79	20·83
Sept. ...	542	432	974	1	4	5	3	3	6	985	85	11·58
Oct. ...	494	342	836	8	9	17		4	4	857	75	11·42
Nov. ...	1157	759	1916	13	6	19	9	3	12	1947	76	25·62
Dec. ...	946	486	1432	11	4	15	9	7	16	1463	75	19·51
Totals (77 weeks)	14293	9280	23573	197	111	308	113	81	194	24075	1280	18·81

TABLE V.

Station	Distance from Sultan Hamud in miles	Passenger Trains			Goods Trains		
		No. of trains exam'd	No. of flies coll'd	Average per train	No. of trains exam'd	No. of flies coll'd	Average per train
Kima	10	3	1	0·3	6	24	4·0
Kiu	19	3	5	1·7	4	10	2·5
Ulu	28	3	3	1·0	7	66	9·4
Konza	36	4	4	1·0	5	15	3·0
Athi River	63	4	—	—	5	8	1·6
Embakazi ... ...,	73	3	—	—	7	30	4·3
Nairobi	79	2	3	1·5	4	5	1·2
		22	16	0·73	38	158	4·16

In this instance, and throughout these observations, *G. longipennis* was the outstandingly predominant species. Another noteworthy feature is that the majority of *G. pallidipes* and *G. brevipalpis* collected on trains in the fly belt were taken between Mtito Andei and Makindu. Furthermore, the graph shows considerable decreases in catches on the Makindu side of Nairobi on the one hand and on the Voi side of Mombasa on the other. The degree of decrease towards the coast is greater than towards Nairobi which suggests that conditions in the former are more unsuitable for, or have a more adverse effect on, *G. longipennis* than in the latter. At the Kiboko river, however, up-trains probably attract still more flies (see also p. 528) and thus augment the numbers carried on to Emali, Sultan Hamud and to the alienated farms beyond.
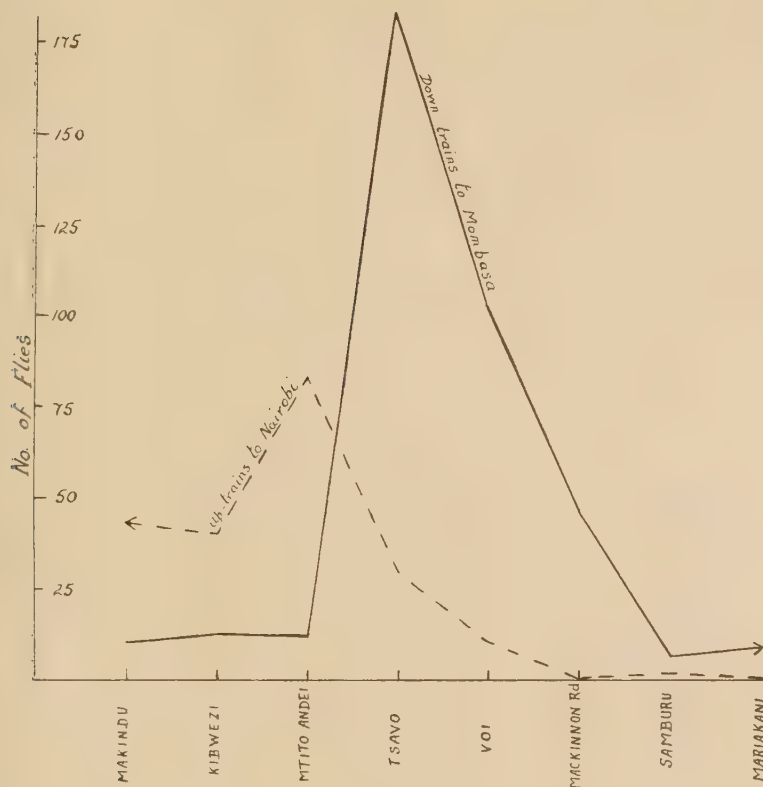
Fig. 1.—Average number of flies per goods train at stations between Mariakani and Makindu.

TABLE VI.

Down-trains.

Station	Distance from Nairobi in miles	Passenger Trains			Goods Trains		
		No. of trains exam'd	No. of flies coll'd	Average per train	No. of trains exam'd	No. of flies coll'd	Average per train
Makindu	119	3	4	1·3	4	41	10·3
Kibwezi	132	4	4	1·0	6	70	11·6
Mtito Andei	164	4	10	2·5	7	80	11·4
Tsavo	194	3	20	6·7	4	743	185·7
Voi	226	3	9	3·0	5	516	103·2
Mackinnon Road ...	267	3	6	2·0	6	276	46·0
Samburu	286	3	17	6·3	5	28	5·6
Mariakani	304	2	2	1·0	2	18	6·0
		25	72	3·0	39	1772	45·4

TABLE VII.

Up-trains.

Station	Distance from Mombasa in miles	Passenger Trains			Goods Trains		
		No. of trains exam'd	No. of flies coll'd	Average per train	No. of trains exam'd	No. of flies coll'd	Average per train
Mariakani	26	2	—	—	3	—	—
Samburu	44	3	1	0·3	8	7	0·9
Mackinnon Road ...	63	3	4	1·3	3	1	0·3
Voi	104	3	9	3·0	5	51	10·2
Tsavo	135	3	9	3·0	6	178	29·6
Mtito Andei	166	4	57	14·3	9	746	82·6
Kibwezi	197	3	13	4·3	12	477	39·8
Makindu	211	3	46	15·3	12	514	42·8
		24	139	5·18	58	1974	34·0

A few searches for tsetses on trains have also been carried out, at intervals, along other sections, and on some branch lines, of the Kenya and Uganda railway. On the Voi-Moshi branch, *G. longipennis* was commonly found on trucks at and near Voi, and in the neighbourhood of Taveta. Only a very small number, however, has hitherto been obtained, between Bura and Mbuyuni on trains travelling west towards Tanganyika and on trains travelling east. *G. brevipalpis* has also been taken at Taveta which is considered to be, for practical purposes, within a fly belt. The possibility of introducing *G. swynnertoni* and *G. morsitans*, potent vectors of human trypanosomiasis, by trains (and road vehicles) from the apparently advanced fly-belt in Tanganyika is not very remote although neither of these two species has yet been discovered on or near the Moshi-Voi road and railway. It is well, however, to bear in mind Swynnerton's comment (1936) on the carriage of tsetses by trains and cars : " When the Shinyanga-Mwanza road ran through a fly belt, flies (*G. swynnertoni*) were carried in fifties and hundreds to forty miles and more into the open country to the north by numerous lorries and cars daily. A tsetse (*G. morsitans*) was recognised by Burtt on a train in Dodoma station nearly 100 miles from the nearest fly belt, and he considers that he has evidence for the view that the relatively recent and isolated Hika fly belt in Manyori, which he investigated, was created by the daily immigration into new country of the great numbers of flies (*G. morsitans*) on the trains. The number of flies which it takes to found a new colony under favourable conditions is unknown ; it would seem from other evidence to be considerable ; but railroads and motor roads passing from actual fly country into potential fly country are at least a very great danger."

With regard to trains entering Kenya from Uganda where *G. palpalis* was at one time suspected of being carried to the settled farming district of Trans Nzoia, searches have so far proved to be negative. Such is the case also concerning trains on the Miwana—Fort Ternan sector of the Nakuru-Kisumu branch line of the Kenya and Uganda railways. *G. palpalis* on the Nyando river and its tributaries is not attracted, apparently, from its riverine haunts by trains which run parallel to the main river and across several of its tributaries.

The situation on the Nairobi-Thika railway branch has not been determined.

Trypanosomiasis.

The three species of tsetse flies commonly found on the Mombasa-Nairobi trains are known to be vectors of animal trypanosomiasis. Laboratory studies at Kabete have shown that each of the three species is capable of transmitting *Trypanosoma*

congolense, T. vivax and *T. brucei*, and that each of these species of trypanosomes will provoke a serious and, often, a fatal disease in some class or other of domestic animals. *G. pallidipes* is readily infected by all three trypanosomes and transmits in the case of *T. congolense* generally and with a particular " strain " of *T. vivax* obtained from Emali, a virulent form of trypanosomiasis which, in the absence of therapeutic treatment, causes heavy mortality in cattle, native sheep and sometimes in goats. *G. brevipalpis* is notably susceptible to *T. brucei* which is often fatal to horses, pigs and dogs. Consequently, a small number of these two species of fly may give rise to a very serious outbreak of trypanosomiasis. The limited number of laboratory trials so far made with *G. longipennis* as a vector of *T. vivax* indicate that the fly sometimes reduces the virulence of a reaction to bovine trypanosomiasis from an acute to a chronic form of the disease. At other times *G. longipennis* will transmit the virulent disease. Its ability to transmit *T. congolense* seems to vary for some reason which has not yet been ascertained. Nevertheless, the presence of one or two specimens of this species alone has been associated with high mortality in two or three valuable dairy herds near Nairobi.

The significance of fly dispersal brought about chiefly by trains is further illustrated by field observations on the incidence of trypanosomiasis in the vicinity of the railway outside the main fly belt. Reliable accounts from early settlers, and reports on investigations by Government officers, refer to periodical outbreaks of the disease as far back as 30 years ago. The recurrence of animal trypanosomiasis from time to time on the same and on other farms bordering the railway not only endorsed earlier evidence but also points to successive importations of the vector. The outbreaks vary very much in frequency and in severity and appear to coincide with the degree and extent of fly dispersal. Outside a fly's normal ranging distance (2–3 miles) from the railway, more especially on the northern side, in the Lower Highlands, they are spasmodic and sporadic ; they occur at irregular intervals and usually involve a smaller number of cattle. Isolated cases are met with more frequently and over a wider field (Nairobi and Ngong). Sometimes, however, the disease may persist in a somewhat latent phase, masked by more or less continuous treatment, but resulting in an accumulative and ultimately, heavy loss. Occasionally, the outbreak may be of a fulminating character especially in herds of milk-producing cattle.

Although detailed records are not yet available, and may not be obtainable in the case of earlier outbreaks, the gravity of the situation may also be gauged by the increasing apprehensiveness of farmers in the settled Lower Highlands and the reluctance of others to occupy land in the relatively fly-free areas of the Coast.

As the distance from the edges of the main fly belt decreases, animal trypanosomiasis becomes more continuous in its incidence, and takes a heavier toll of cattle. This is the experience on a stock-farm at Emali, the records of which provided the figures, relative to the situation, given in Table VIII.

The total numbers of cattle (column 8) on the farm include calves and young animals, cows, bullocks and bulls. The animals are of native breed (Boran and Boran cross) and, perhaps not insignificant, of a beef rather than high milk-yielding type. From 20 to 32 are utilised as work-oxen. It will be noted that the recorded number of infections is relatively high except in the year 1946 which is for the first five months only. There is also some suggestion, not indicated in the Table, of a seasonal fluctuation which may be related to an apparent periodicity in the numbers of flies collected at the Sultan Hamud de-flying station (see last column, Table IV). The mortality is considerably less than the number of positive cases, a result probably brought about by early diagnosis and treatment, constant personal attention by the farmer, good feeding, and the absence of strain and exertion associated with milk-production and heavy draught.

A valuable bull was lost from *T. congolense* infection ; and a horse and dogs died of *T. brucei*.
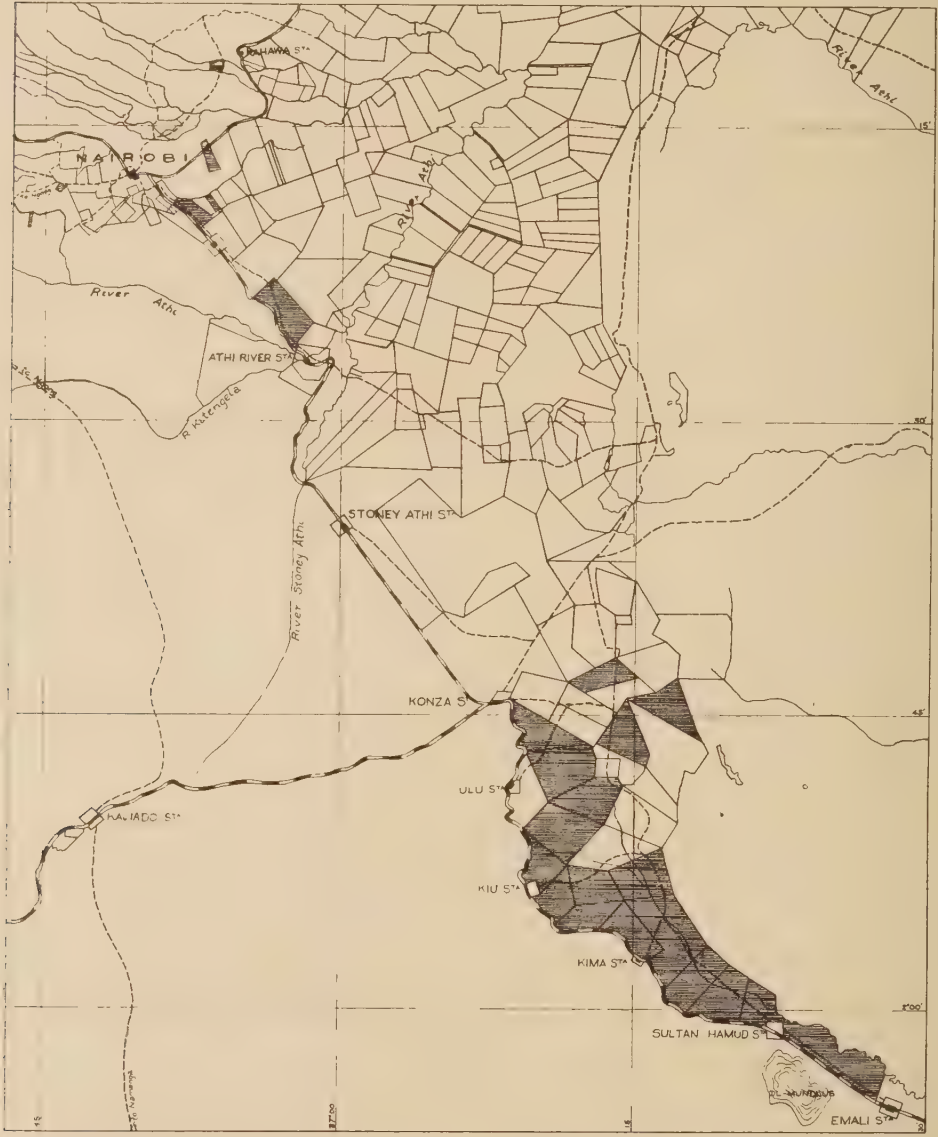
TRYPANOSOMIASIS IN THE LOWER HIGHLANDS OF KENYA COLONY

MAP II.   Shaded areas represent farms where outbreaks of animal trypanosomiasis
have been recorded and where quarantine has been imposed

TABLE VIII.

Year	T. congolense	T. vivax	T. congolense and T. vivax	Un-identified trypano-somes	Total infections	No. of deaths	Total No. of cattle
1941 ...	25	7	5	5	42	23	596
1942 ...	46	36	2	5	89	32	695
1943 ...	35	5	1	1	42	15	683
1944 ...	153	26	10	5	194	50	872
1945 ...	38	14	0	2	54	21	849
1946 ...	6	0	0	0	6	6	842
Total for 6 years ...	303	88	18	18	427	147	

The position with regard to animal trypanosomiasis among herds of African-owned (Wakamba and Masai) cattle which often graze over the country between the farm at Emali and the fly-belt is not known in detail. A herd of Masai cattle at the foot of Olmundus was, on one occasion, found to contain many infected cattle, and several dying beasts were proved to be suffering from trypanosomiasis (*T. congolense* and *T. vivax*). Many other herds roam over the country stretching from Emali to Simba and sometimes as far as the Kiboko river but the cases of trypanosomiasis seen at one time or another cannot definitely be associated only with flies carried by trains. The allocation of specified grazing grounds in this area for native cattle, or the establishment of settlements including stock will, nevertheless, expose animals to infection as much as, and probably more than, in the case of occupied farms farther up-country and at a greater distance from the main fly-belt.

The situation on the coastal side of the fly-belt is no doubt very much the same; but the likelihood of an increase in the volume of road (and rail) traffic to and fro between Mackinnon Road and Mombasa may aggravate the state of affairs and cause heavier losses among native stock in the Samburu-Mariakani marginal zone and in the Coastal sector.

Discussion.

The observations set out in the foregoing sections lead to the conclusion that, while the tsetse infestation in the main fly belt remains in its present state, trains will continue to convey flies and contribute to the spread of animal trypanosomiasis to farms and grazing grounds outside the permanent fly belt itself. Trains carrying tsetses will again in the future constitute an obstacle and a menace to successful settlement and development unless some highly effective method of dislodging the flies is devised. It is a problem which will, if it does not already, concern other territories in East Africa such as Nyasaland, Tanganyika and Portuguese East Africa.

Bush-clearing on individual farms and on other land outside the area of natural fly infestation, in the part of Kenya Colony under consideration, provides no protection against the importation of flies, but it discourages wide dispersal on the part of the tsetses carried beyond their usual habitat. It reduces the amount of shelter required by the flies, and increases the unfavourable conditions associated with the altitude. It restricts activity, and assists in the speedy destruction of large numbers of flies. It does not safeguard the farmer from losses due to sporadic outbreaks of animal trypanosomiasis for which he is liable to be penalised by quarantine regulations under the *Disease of Animals Ordinance.*

The removal of woody vegetation on both sides of the railway line which traverses the 177 miles of the fly belt cannot readily be undertaken, and it would take several years to achieve. Much of the country is waterless for long periods and long stretches of it are at present considered to be unsuitable for crop production. In some sections, the ground is bare partly, no doubt, on account of the poverty of the shallow soil and, partly because of the dryness and of the dense growth of low desert shrubs. The country between Tsavo station and Mtito Andei, a stretch of about 30 miles, is reserved as a National Game Park. The human population is extremely low ; most of the land near the railway is uninhabited.

The settlement of people, with a view to ranching as a main proposition, must of necessity be a gradual process in such a strip of country. In the meantime, and while projects of reclaiming the fly-infested country are under way, it is at least highly desirable to protect stock-farmers in the marginal sectors by preventing the dispersal of tsetses by trains. The de-flying of trains is an essential supplementary, if not an initial, step. Other measures of fly control would thereafter proceed more effectively and satisfactorily.

Some localities of tsetse concentration appear to be suitable for cultivation. At the Kiboko rivers, where regular fly-patrols are operating, the high density of *G. pallidipes*, *G. longipennis* and *G. brevipalpis* can almost certainly be reduced by discriminative clearing of bush, by controlled burning and by trapping in selected foci of infestation. Incidentally, the use of traps for *G. pallidipes* (Lewis, 1941) is comparable, to some degree, to the " catching-out " of *G. palpalis* by fly-boys (Symes & Vane, 1937 ; Glasgow & Duffy, 1947) in isolated blocks of uncleared forest. Controlled burning seems to have been effective in reducing *G. longipennis* infestation in the Makueni location of the Machakos district where fallen trees and logs—favourite breeding-places of the species—were also destroyed by fire. The removal of dense undergrowth and thinning out of some trees has resulted in the disappearance of *G. brevipalpis* from a fairly wide area. Similar measures to be adopted at Kiboko will not only bring about a reduction of the fly infestation, but will also affect the numbers of flies attracted, at this point, to passing trains. A recent series of observations on the railway line in this locality is of further interest. Fly-boys, under personal supervision of the author, were posted at intervals of 200 yards, along the railway from 6.15 p.m. to 7.10 p.m. to cover the period of the evening activity of *G. longipennis*. A few *G. pallidipes* were caught just before dusk. Then *G. longipennis* appeared in fairly large numbers, and the fly boys caught from 8 to 16 flies each up to 6.50 p.m. when a train passed. Not one fly was caught afterwards. On the following night, the train passed before 6.30 p.m., and catches of *G. longipennis* continued up to 7.5 p.m. The implication is that the flies on the first night were swept up by the passing train, whereas, on the second night, the train passed through the sector before the activity of *G. longipennis* had reached its peak.

Other localities of fly concentration where bush-clearing is contemplated are at and between Kibwezi and Masongaleni, between Ndi and Mtito Andei, and on the Tsavo river.

The de-flying of trains is a pre-requisite of successful tsetse control in these somewhat isolated centres where bush-clearing as a supplementary measure will provide for the settlement of relatively small communities of people who in due course can expand over a greater area of land, introduce goats, sheep and, later, cattle—a scheme similar in some respects to the Sleeping Sickness Settlements which have proved successful in many parts of Tanganyika (Fairbairn, 1943).

It has been shown in this paper that large numbers of tsetses are taken from trains by fly-boys posted at stations outside the fly belt especially where the stops are reasonably long, but that many flies escape capture at these stations and are carried

farther up-country. Still larger numbers, presumably, are carried on night trains which hitherto have not been subject to de-flying by hand, except the inside of passenger trains.

A more efficient method of dislodging the tsetses on all trains is therefore required. Such a method has not yet been devised ; but the gear used for washing carriages in some railway and motor-bus depots in England and probably in America suggests a design of an appropriate spraying apparatus. Several colleagues who have been consulted have suggested a similar outfit : namely, a hoop of pipes which lead to a number of spray nozzles fitted and arranged so as to ensure an even and complete coverage of coaches and trucks. The spray in the form of very fine particles, mist or aerosol should be conveyed to and contact flies which are frequently seen on the sides, at the ends, and on the undercarriage of trucks and coaches. Over a period of 52 weeks, the de-flying teams collected 5,080 tsetses from the sides and ends of trucks, 14,986 from the undercarriage, and 642 from engines. A compressor plant, easy to operate and maintain by a trained African staff, should be portable in order to enable its removal from station to station as the centres of concentration, from which flies attracted to trains, are eliminated by bush-clearing or other measures of tsetse control.

Furthermore, spraying of trains while travelling—at low speed—is desirable in order to overcome difficulties connected with holding-up railway traffic on which there is an increasing demand in East Africa. Convenient sites would be near railway stations where trains often slow down to about 5 to 10 miles an hour. The draught created by a fast-moving train would, thus, also be reduced necessitating only a short tunnel or shed to prevent the spray from being swept away from the train.

The application of an insecticidal smoke is being considered. An essential property of an insecticide, in this case, is that of a quick " knock-down ". The primary object of the de-flying of trains is to remove the flies, and to prevent their being carried to stock areas normally, or rendered, free of tsetses. The incorporation of pyrethrum extract is, therefore, indicated—a product obtainable locally. A lethal property would, of course, be of advantage. It might be obtained either by increasing the percentage of pyrethrin or by including DDT or BHC in the spray or smoke. The residual effect of a deposit of the spray on the vehicles is of secondary importance. The trucks and coaches of a train are often changed, washed or painted ; and they collect a considerable quantity of fine dust which forms a coat that would cover the insecticidal deposit. Nevertheless, a residual lethal insecticide is not without value. The cost of the insecticide, however, is of considerable importance as every train from the fly belt has to be sprayed, which constitutes a recurrent expenditure. According to the East Africa Railway authorities, a daily average of 5·5 trains are run, at present, from Mombasa to Nairobi ; and it is envisaged that the average will increase to over 7·7 per day. The length of these trains is often from about 1,200 ft. to 1,700 ft. The quantity of the spray required is likely to be large. Two stations for de-flying trains are contemplated ; one at Makindu or Kibwezi for trains from Mombasa to Nairobi, and another at Mackinnon Road for trains from Nairobi to Mombasa. The railway line is a single track.

The daily passenger train to the Coast reaches Makindu soon after 9 p.m. whereas the up-country train from Mombasa reaches Mackinnon Road shortly after dusk. Spraying the compartments, corridors and toilet could conveniently be carried out at these stations and the gauze screens for windows be set in position before the trains enter the main fly belt. A definite system of spraying at these stations, specified on notices in the compartment would help to destroy tsetses entering passenger trains and reduce the transport of mosquitos. Passengers would be more likely to appreciate the purpose of spraying and the duties of the attendants.

Road de-flying posts may also be necessary if the motor traffic between Mombasa and Nairobi increases, and especially if vehicles travel in quick succession or in convoys ; and after sunset when *G. longipennis* in particular seems most active.

Incidentally, this apparently peculiar activity of *G. longipennis* when the flies appear suddenly, as it were, at dusk and for a short period afterwards (Lewis, 1942a) needs further investigation in view of the observation that this species seems to be attracted to trains travelling through the fly belt during the day as readily as to trains travelling at night. Female *G. longipennis* are nearly as common, on the trains, as male flies whereas catches by patrols at dusk comprise practically entirely male *G. longipennis*.

It is submitted that the evidence adduced in this account of investigations so far carried out warrants an early and active approach to the problem of de-flying trains infested by tsetses. It has already been stated that the three species of flies carried by trains in Kenya Colony are capable of being infected and of transmitting trypanosomes pathogenic to farm animals, as laboratory tests at Kabete and elsewhere have shown. Field observations also have shown that cattle cannot remain long in the presence even of a few *G. pallidipes* without becoming infected with *T. congolense* or *T. vivax* or both ; and in the Emali area *T. vivax* is often equally or more virulent in cattle than *T. congolense*. Laboratory experiments, the results of which will be published later, confirm this last field observation. *G. longipennis*, the most abundant species on trains, is an experimental vector of *T. brucei* as well as of *T. congolense* and *T. vivax*. Field observations, however, indicate that it may not always harbour pathogenic trypanosomes either because it is not a highly suitable host, as *G. pallidipes* is, or because in its normal habitat it prefers to feed on game, rather than on farm animals. These two aspects of the problem are now being investigated.

Summary.

Railway trains running through tsetse-infested country between Mombasa and Nairobi in Kenya Colony carry *G. longipennis*, *G. pallidipes* and *G. brevipalpis* on to stock-farming areas beyond the natural limit of the fly belt, thus being largely responsible for outbreaks of animal trypanosomiasis and a reluctance on the part of European, Asian and African settlers to develop the land, affected by this form of fly dispersal, to its full capacity.

The country through which the trains travel is described together with an account of a survey of the fly distribution.

Tabulated figures are given of the monthly totals of tsetses collected from passenger and goods trains by teams of fly boys posted at Emali and Sultan Hamud railway stations about 17 and 25 miles respectively outside the fly belt towards Nairobi. More than 24,000 flies were collected over a period of one year and many, not collected by the de-flying teams, were carried up-country even as far as Nairobi, 101 miles from the fly belt.

Tsetses were collected from inside passenger trains, but very much larger numbers were taken from the outer surfaces and undercarriages of goods trucks.

Reference is also made to the rôle of motor traffic, especially when in convoy, in the dispersal of tsetses.

The incidence of animal trypanosomiasis along the line of the railway is dealt with and the association of the disease with tsetses carried by trains is supported by results of laboratory experiments on the transmissibility of *Trypanosoma congolense*, *T. vivax* and *T. brucei* by the three species of flies concerned.

Bush-clearing on stock farms outside the fly belt will not protect the cattle from infection carried by flies brought by trains but bush-clearing in specified localities of fly concentration in the infested country is recommended as a measure to reduce the

numbers now attracted to trains. It is considered that the spraying of trains will bring considerable relief to farmers ; but, so far, no suitable spraying apparatus has been devised nor has a reasonably cheap insecticide been found for this purpose.

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PUPATION HABITS OF SHEEP BLOWFLIES IN RELATION TO PARASITISM BY *MORMONIELLA VITRIPENNIS*, WLK. (HYM., PTEROMALID.).

By G. C. Ullyett,
*Commonwealth Bureau of Biological Control.**

The three main species of flies inhabiting carrion in South Africa, namely, *Lucilia sericata*, Mg., *Chrysomyia chloropyga*, Wied., and *C. albiceps*, Wied., exhibit marked differences in pupation habits which have a direct bearing upon their relative susceptibility to parasitism during the pupal stage. This is particularly so where *Mormoniella vitripennis*, Wlk., is the predominant parasite species, since this Pteromalid is unable to reach puparia which are formed in the more protected situations such as beneath a layer of sand. The following experiments were made in order to determine what proportion of the population of each of the three blowfly species was immune from the attack of this parasite, and hence the relative survival values, after competition for food among the preceding larval populations, had reduced the general level of the progeny.

Depth of Pupation.

Method.—Sifted river sand was put into a breeding box 12 ins. sq. by 12 ins. deep, the sides of which were graduated in half-inches. The box was filled to within 3 ins. from the top and was closed with a sliding glass lid. Ventilation holes were provided in the sides of the box, these being covered with fine-mesh wire gauze. Fully-grown larvae were placed on the surface of the sand, 500 to a box. Four replications were set up for each species of fly, the boxes being kept at 80°F. until the puparia had been formed. The sand was then removed in half-inch layers and sifted, the number of puparia in each successive layer being recorded.

Results.—In each individual species, the four replications gave remarkably close agreement. The depth of pupation in the three species is compared in Table I, the figures being the average of the four replications.

TABLE I.
Depth of pupation in sand of three species of blowflies.

Depth of pupation in inches	L. sericata		C. chloropyga		C. albiceps	
	No. of pupae	Per cent.	No. of pupae	Per cent.	No. of pupae	Per cent.
On surface	5·75	1·13	285	57·00	493·67	98·73
½ ...	54·75	11·05	132	26·40	5·33	1·07
½–1½ ...	271·00	54·66	77	15·40	0·67	0·13
1½–2½ ...	86·13	17·38	6	1·2	0·00	—
2½–3½ ...	58·25	11·75	0	—	0·00	—
3½–4½ ...	18·00	3·63	0	—	0·00	—
4½–5½ ...	2·00	0·40	0	—	0·33	0·07
Totals ...	495·88	100·00	500	100·00	500·00	100·00

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It is evident that, where burial beneath the surface of the sand constitutes immunity from attack by parasites, as is the case with *Mormoniella, Lucilia sericata* is very adequately protected. In this species, 98·87 per cent. of the puparia are formed in the sand while, in contrast, the majority of the *Chrysomyia* puparia occur on the surface. In the case of *C. chloropyga* the proportion thus exposed is 57 per cent. ; with *C. albiceps* it is 98·73 per cent. Thus there is an initial tendency for *Lucilia* larvae to seek for a protected situation in which to pupate, a tendency which seems almost totally lacking in the other two species. This confers a degree of immunity from parasite attack on *Lucilia* which offsets, to a great extent, the fact that this species is a preferred host of the parasite. It is interesting to note that the degree of protection sought by the larvae of the three species in the present experiment is in direct proportion to the preference shown by the parasite for the species as hosts.

Pupation Sites.

Method.—As an extension of the above tests, the same three species were used, but in the present instance the larvae were allowed to develop to maturity on pieces of lean beef contained in galvanised iron dishes measuring 4 ins. sq. The dishes were placed over sand in breeding boxes which were 18 ins. sq. by 4 ins. deep and the larvae allowed to crawl off the meat and pupate at will. Two separate series were set up, one in which 100 larvae were present on 140 grammes of meat, the other in which there were 1,000 larvae on pieces of meat of the same size. The object of the two series was to see whether population density affected the results. After puparia had been formed, the sand was sifted as before and the location of the puparia was recorded in each case.

Results.—In the present series, additional sites for pupation were available owing to the presence of both meat and dish in the breeding box. These gave varying degrees of protection to the pupae from the attack of *Mormoniella*. The sites and their rating as protective situations are shown in the Tables giving the summaries of results. Four replications in each series and for each species were carried out. These gave very consistent results.

TABLE II.

Pupation sites of blowfly larvae : 100 larvae per 140 gm. meat.

Position of puparia	Per cent. pupation of			Relation to attack by *Mormoniella*
	L. sericata	*C. chloropyga*	*C. albiceps*	
In the dish	0·48	9·39	21·02	Exposed.
In the meat	0·00	1·46	46·50	Exposed.
Under the dish	25·42	35·49	18·78	Partially protected.
On the sand	15·68	34·03	13·06	Exposed.
In the sand	58·43	19·62	0·64	Completely protected.

From Tables II and III it is clear that the larvae of the three species of blowflies have very marked preferences for sites in which to pupate and that these are chosen consistently and without any reference to the density of the larval population present in the environment. The great majority of the *Lucilia* larvae burrow into the sand while the bulk of the remaining two species form puparia without doing so. Again, this shows a close approximation to their order of suitability as hosts for *Mormoniella*.

C. albiceps, as the least suitable and least favoured, is found in the most exposed situations, while *Lucilia* which is the most suitable, seeks the most protected site for pupation. *C. chloropyga* occupies an intermediate position in both respects. This is made clear by the summary in Table IV.

TABLE III.

Pupation sites of blowfly larvae : 1,000 larvae per 140 gm. meat.

Position of puparia	Per cent. pupation of			Relation to attack by *Mormoniella*
	L. sericata	*C. chloropyga*	*C. albiceps*	
In the dish	2·69	2·30	10·26	Exposed.
In the meat	1·16	18·04	35·06	Exposed.
Under the dish	5·01	38·42	23·21	Partially protected.
On the sand	8·78	34·98	29·47	Exposed.
In the sand	82·35	6·26	2·00	Completely protected.

TABLE IV.

Relationship of blowfly pupation to parasite attack.

Protection from parasitism	Percentage of pupae of		
	L. sericata	*C. chloropyga*	*C. albiceps*
Protected completely	70·39	12·94	1·32
Partially protected	15·21	36·96	21·00
Exposed	14·40	50·10	77·68

Discussion.

In the struggle for existence induced by intense competition for food between the larval populations of blowflies on carrion, *Lucilia* sets out with certain initial disadvantages. It is smaller in size than the other two species and hence is more liable to be crowded off the medium. It is also very susceptible to the predatory habits of *C. albiceps* and a very marked reduction in the population of *Lucilia* takes place where these two species occur together on the same carcase. To offset these disadvantages, there are certain peculiarities in the growth of *Lucilia* populations which serve as adaptations to adverse conditions of overcrowding and which tend to lead to the production of larger surviving populations than would otherwise be the case. These adaptations are described elsewhere.* The most noteworthy of these phenomena is the relative rate of growth of *Lucilia* larvae as compared with those of the species of *Chrysomyia*. Basically, there should still be a larger surviving population of *C. albiceps* than of *Lucilia* where these two species come into conflict for the same amount of food medium, since this can be demonstrated experimentally where competition for a common food supply is the only limiting factor. This is due largely to the predatory habits of *C. albiceps*. Records of actual field populations, however, show that this is not necessarily, and indeed not usually so, but that the position of the two species in nature is just the reverse. Obviously it is necessary to look for causes other than straight competition for food to explain this.

Although competition is an important and, under certain conditions, an efficient factor in reducing the total number of individuals in the larval populations of sheep blowflies, a significant proportion of these populations nevertheless usually survives to form puparia. It is then that they become exposed, to a greater or less extent, to the attacks of parasites and predators. During the egg and larval stages, which are both comparatively short, little or no parasitism occurs. The pupal stage is longer and a number of different species attack it, the dominant one being *Mormoniella vitripennis*, which is usually very common in the field and will give a very high percentage parasitism of puparia exposed to its attack. For these reasons, the remarks made here apply to parasitism by *Mormoniella* and not necessarily to that by other species, some of which have slightly different habits.

Puparia which are formed beneath the surface of the soil are inaccessible to *Mormoniella* females since the latter are apparently unable to penetrate to any depth below the surface. Thus, all pupae which occurred in the sand in the experimental boxes were immune to attack. Similarly, puparia formed underneath the meat dish, where it rested on the sand, were also to a large extent protected by their position. Hence the choice of a site for pupation by the fully-grown blowfly larvae determines the susceptibility of the resulting puparia to attack by the parasite.

From the experimental data presented above, it is clear that *Lucilia* is very largely immune from the attacks of *Mormoniella* when the larvae are able to achieve the positions for which they normally and instinctively aim. As already mentioned, *Lucilia* is the most-favoured host of this parasite and, were it not for this tendency to choose protected situations for pupation, the population which managed to survive competition for food would usually be almost completely killed off by parasitism. As it is, the majority of the individuals actually survive to produce adult flies and the field population is maintained at a comparatively high level. Normally, this level is sufficient to produce progeny which can occupy the bulk of the available carrion in any given environment.

On the other hand, the *Chrysomyia* species, while they are apparently not as suitable as hosts for the parasite, are more exposed to its attack. *C. chloropyga*, which is more suitable than *C. albiceps*, is more protected than the latter species but the bulk of its population is still largely exposed to parasitism. *C. albiceps* is almost completely vulnerable to attack, since the majority of its larvae pupate in the remains of the carrion and the puparia are so formed that approximately one-half to three-quarters of the body protrudes into space.

Normally, where a choice is offered between *Lucilia* or pupae of *C. chloropyga* and *C. albiceps*, *Mormoniella* will choose one of the first two species, *Lucilia* being the first choice (J. S. v.d. Merwe, unpublished data). The puparia of *C. albiceps* are apparently not suitable for the parasite progeny since they are tough and a large proportion of the emerging adult parasites are unable to penetrate the skin and consequently die within the puparium. Since the majority of the *Lucilia* pupal population is protected from attack and a significant proportion of the *C. chloropyga* population is similarly removed from its sphere of action, *Mormoniella* is forced to accept the more available *C. albiceps* puparia.

This undoubtedly happens under field conditions and, although increase in the parasite population is adversely affected by this use of an intrinsically unsuitable host, the *C. albiceps* population must be very considerably reduced by the results of parasitism. The field records available for these three species of blowflies show that the population of *C. albiceps* emerging from carcases exposed throughout the year is always very much smaller than that of either of the other two species (Ullyett, *loc. cit.*). In view of the predatory habits of *C. albiceps* and of the known results from experimental mixed populations of larvae, this is the opposite to what would be expected from the effects of competition for food pure and simple. It would

seem, therefore, that the comparatively small surviving populations of adult *C. albiceps* must be put down to the relative accessibility of the species of blowfly pupae to parasite attack.

From the general view-point of natural control, this phenomenon is disadvantageous since *C. albiceps*, a secondary fly with a predatory larvae, is an important factor in the control of *Lucilia*, a primary species of sheep blowfly. The important pest species is favoured, among other things (*vide* Ullyett, *loc. cit.*), by its pupation habits, both directly and indirectly, and on this account can maintain a comparatively large population in the field. In nature, *Lucilia* is known to pupate largely in the soil at the base of the bushes growing near the carrion.

Summary.

The depth and habits of pupation, shown by the three main species of sheep blowflies (*Lucilia sericata, Chrysomyia chloropyga*, and *C. albiceps*) are discussed in relation to parasitism by the Pteromalid, *Mormoniella vitripennis*.

Experimental series showed that the majority of *Lucilia* larvae pupate in situations which are inaccessible to the parasite, a result which agrees with field observations ; whereas the two species of *Chrysomyia* have a large proportion of their pupal populations exposed freely to attack by the parasite. The degree of protection thus sought by the larvae appears to be directly proportional to the suitability of the puparia of the species as hosts for the parasite.

Because of this difference in pupation habits, a large proportion of the *Lucilia* population which survives the competition for food and the predatory attacks of *C. albiceps* on the carrion will produce adult flies ; whereas the more exposed *C. albiceps* and *C. chloropyga* populations will be further reduced and in a proportionate manner by parasitism. This explanation is put forward as one of the main reasons for the comparative success shown by field populations of *Lucilia* and the unexpectedly small field populations observed in the case of *C. albiceps*.

It is pointed out that this phenomenon is a disadvantage where natural control of the primary flies is concerned since *C. albiceps* is an important controlling factor in larval populations of *Lucilia*.

THE MATURATION OF THE OVARIES AND THE RELATION BETWEEN WEIGHT AND MATURITY IN *LOCUSTA MIGRATORIA MIGRATORIOIDES* (R. & F.).

By John Phipps, M.Sc., D.I.C., F.R.E.S.

Entomologist, Medical Department, Tanganyika Territory.

The work described in this paper was carried out at the Imperial College of Science, partly in London and partly at the Field Station, Silwood Park, Berks.

The objects of the investigation were to describe the process of maturation of the ovaries, to clear up the question of the relation between copulation and sexual maturity, and to determine whether any relation exists between weight and sexual maturity. It is well known that the ovary of the female locust is undeveloped for some time after the final moult, and hitherto the acquirement of maturity has been measured only by the time of the first oviposition. This is not entirely satisfactory, since there is no evidence that the eggs are laid as soon as they are ripe.

Pospelov (1934), dealing with the relation between copulation and sexual maturity, states that females do not become sexually mature if they are not allowed to copulate, while Hamilton (1936) records that virgin females oviposited and examples of parthenogenesis in *Locusta migratoria*, L., and other species are quoted by Uvarov (1928). The accounts given are summarised below, and an attempt is made to explain the discrepancy.

Material and Methods.

Hoppers in the fifth instar were originally obtained from the Anti-Locust Research Centre at the British Museum (Natural History) ; later, locusts were bred at the Imperial College Field Station. As the number of locusts maintained increased, it was necessary to make use of cages of several different types. The chief types used were as follows :—

(1) Two wire gauze cages, 12 in. × 12 in. × 12 in., were heated by means of a small electric heating pad buried in the sand. The loss of heat through the sides when the cages were in the laboratory was such that in order to maintain the air temperature in the cage at 30°C. it was necessary to raise the sand temperature to 60°C. The cages were, therefore, installed in a constant temperature room at 25°C. but even here it was necessary to keep the sand temperature too high for oviposition. About twenty locusts were confined in each of these cages.

(2) Eight 7-pound glass jars, each with about 3 ins. of sand in the bottom, were placed in an incubator maintained at about 34°C. Four locusts were kept in each jar. This method was very successful, and the locusts oviposited freely.

(3) A glass tank 14 in. × 14 in. × 12 in. was used for keeping larger numbers of locusts, and was especially successful for breeding. It was heated by a heating mat buried in the sand, and also by an electric bulb suspended in the tank to maintain the air temperature. It was found that in the absence of the bulb the sand had to be maintained at too high a temperature, as in the case of the wire gauze cages already described. Locusts oviposited readily in the tank, and it was noted that this was usually close to the sides of the tank, so that the tip of the abdomen of the ovipositing female could often be seen through the glass.

(4) Three cases of expanded rubber, 25 in. × 25 in. × 25 in., were each fitted with a glass lid and heated by heating mats or bulbs below the case. A hole in the bottom

of the case conducted hot air to the inside. This method permitted the breeding of much larger numbers of locusts, but was put into use only towards the end of the investigation, when many of the results had already been obtained.

As the experiments were not designed to investigate the influence of temperature or humidity on maturation, no great effort was made to keep conditions very constant. Temperature was maintained between 32 and 37°C. and humidity at about 50–70 per cent. The provision of fresh grass several times a day kept the humidity fairly high, and at the same time caused considerable variation, but as long as the locusts grew satisfactorily in the hopper stages and showed steady weight increases as adults, it was assumed that conditions were favourable. Generally mortality was not high and with abundant fresh grass cannibalism was infrequent, even with considerable crowding.

The first set of experiments consisted of periodic dissections of female locusts, some mated, some unmated, to provide data for a description of the process of maturation in both cases. During this period it was difficult to keep the locusts supplied with grass, and what could be obtained was of poor quality. The probable significance of this is referred to below.

The second set of experiments consisted of keeping and regularly weighing a number of locusts, some mated and some unmated, and recording oviposition. All the locusts were killed and dissected at the end of about three weeks, and the condition of the ovaries noted.

Measurements of elytron and femur length were made in accordance with the decisions of the Fourth International Locust Conference, 1936, in order to estimate the phase of the locusts used. The E/F ratio was found to be 2·0, whence it may be concluded that they belonged to the *transiens* phase, approaching *gregaria*. The calculation was based on measurements of 50 females, and may be compared with the value of 1·931 obtained by Duarte (1938), and also with the value of 2·27 given by Uvarov (1928) for *L. migratoria migratorioides*, R. & F., from Nyasa and Lagos. Apparently Duarte's locusts were bred under rather more crowded conditions than those obtaining in most of the experiments described here.

Ages of locusts were recorded in days from the date of the last moult into the adult stage. This moult is referred to as " emergence ". In the weighing experiments a system of marking was used which made it possible to distinguish individuals and to keep a complete record of each. Marks were made with cellulose paints of different colours in four positions on the prothorax. After some trials, the marks were confined to the sides of the prothorax, since it was found that marks made in the mid-dorsal line frequently disappeared, being probably rubbed off by the males during the prolonged copulation which is usual in locusts.

Maturation of the Ovaries.

General account.

The structure of the ovaries of locusts has been described by Uvarov (1928). The ovarioles are numerous and are of the panoistic type. The egg nearest to the oviduct, which is therefore the first to be ovulated, is referred to for convenience as egg_1, and that above it as egg_2.

The ovaries of newly emerged adult locusts are small and white, and it is often difficult to distinguish the individual ovarioles amongst the mass of tracheae (fig. 1). The ovary shows little change in appearance for some days during which time the fat body shows a considerable increase in size. Measurements of the eggs show, however, that some growth is occurring during this period. After about seven days the eggs begin to grow rapidly and the appearance of the ovary is considerably changed. This change is due chiefly to the growth of egg_1, which grows much more rapidly than egg_2.

Each egg rudiment, in fact, grows more rapidly than the one above it, so the whole ovariole lengthens and becomes more conspicuous.   The ripe egg, by now about 7 mm. long, is finally shed into the oviduct, and the empty follicle which had contained it shrinks rapidly.   At this time the new egg_1 is only about 2 mm. in length and the ovarioles have the appearance of small white appendages of the greatly swollen oviduct.

Shortly after ovulation there appear, in the stalks of some at least of the ovarioles, structures which appear red by reflected light, and which are often quite obvious to the naked eye as small red specks during dissection.   Under magnification it is apparent that the ovariole stalk contains a mass of red coloured tissue, which appears black when viewed by transmitted light.   These masses of tissue are referred to as *corpora lutea*. Vel'tischev (1941) recorded *corpora lutea* in *L. m. migratoria*, and believed them to indicate that ovulation had occurred, but the present investigations showed *corpora lutea* to be present in some ovarioles in a few individuals which had not oviposited.   In such cases they were always associated with degeneration of egg_1 in the ovarioles concerned.   It appears that the formation of a *corpus luteum* is associated with degeneration, either of an egg in the follicle or of an empty follicle after ovulation.   *Corpora lutea* fade and disappear after a few months preservation in alcohol.

Roonwal (1945) noted the occurrence of these structures in *Schistocerca gregaria*, Forsk., and also noted that some eggs have a reddish tinge on the chorion, which he suggests is acquired whilst passing into the oviduct.   This is likely in view of the author's own observations (1949) on British grasshoppers, where it has been clearly seen that the *corpus luteum* forms a ring at the junction of ovariole and oviduct, through which the eggs must pass.   It appears doubtful, however, whether this red pigment has any phase significance, as Roonwal suggests, since red *corpora lutea* are said to occur in both *solitaria* and *gregaria* in *Locusta migratoria* while yellow *corpora lutea* occur in the British grasshoppers.

Stages in Maturation.

The process of maturation of the ovaries is clearly a continuous one, but in analysis of results, and particularly in relating maturity to weight, it was found convenient to distinguish four stages, similar to those used by Nüsslin (1927) for Coleoptera and by Bolduirev (1929) for *Locusta migratoria migratoria* (L.). Definitions of these stages, which involve a more detailed account of maturation, are now given.

Stage I.   Immature.   The ovary is small and white. Egg_1 varies in length from 0·3 mm. (in newly emerged adults) to 1·3 mm.   The oviduct is small, its walls not thickened (fig. 1).

Stage II.   Pre-reproductive.   The ovary as a whole is larger and the fat body is well developed. Egg_1 has a definite yellow colour in fresh material, and varies in length from 1·3 mm. to 2·5 mm. (fig. 2).

Stage III.   Reproductive.   The ovary is very large.   The fat body reaches its maximum development in early stage III ; later it is much reduced and where the eggs are fully developed only a few shreds of it remain. Egg_1 appears almost fully formed in comparison with the much smaller egg_2, and ranges from 3·0 to 7·0 mm. in length.   The oviduct is swollen with secretion, especially in those in which egg_1 is largest (fig. 4).

Stage IV.   In this stage are included all individuals in which ovulation has occurred, irrespective of whether the eggs are retained in the oviduct or have been laid.

In those in which the eggs are still in the oviduct, what is now egg_1 varies in length between 1·2 mm. and 3·0 mm.   Only one individual was found with eggs in

the oviduct in which egg_1 was 3·0 mm. long, usually it is much smaller (figs. 6, 7). The average length is given and discussed later. The largest empty follicles observed were 1·6 mm. long, while the smallest were already reduced to the normal ovariole stalk.

In those individuals which have oviposited the walls of the oviduct are thickened and pigmented. egg_1 varies in length from 2·3 mm. to 7·0 mm., thus having about the same range as in stage III. Such individuals are recognised as stage IV by the presence of *corpora lutea* (figs. 5, 6, 8), but care must be taken not to include those individuals where the *corpora lutea* are associated with degenerating eggs. It is probably not possible to assign any locust to stage IV with complete certainty on inspection of the ovaries, but in view of the rarity of egg degeneration, the proportion of errors in a large sample will not be high.

There is little evidence to indicate how long the eggs may be retained in the oviducts. As noted above, the empty follicles are never very large, and they probably shrink rapidly immediately after evaluation, so that variation in the size of these follicles cannot give information as to the time which has elapsed since ovulation. In the weighing experiments, however, it was found that the weight of a female increased steadily up to the time when she oviposited, without any long period of constant weight which might be expected if the eggs were retained in the oviduct for some time. It appears probable that oviposition usually occurs within a few hours of ovulation.

Number of ovarioles.

Bolduirev (1929) noted that in *Locusta migratoria* the number of ovarioles varies in different individuals and in the right and left ovaries of the same individual and also that some ovarioles always remain small. Roonwal (1946) noted the same in *Schistocerca gregaria*, but found no asymmetry in hoppers. He found some ovarioles smaller than others in fourth-stage hoppers and suggests that reabsorption of these small ovarioles later leads to asymmetry.

It has also been noted by Mrs. M. J. Richards, to whom I am indebted for the information, that pods laid by old females tend to produce fewer nymphs than those laid by young ones. This may be due to the pods containing fewer eggs or to reduced fertility.

The occurrence of small ovarioles was noted early in the present investigation. It was most obvious where egg_1 in the normal ovarioles was 4–5 mm. long. In such small ovarioles egg_1 is not much larger than in a Stage II ovary, and is white or only faintly yellow. These small ovarioles often showed *corpora lutea*, so either they had produced eggs or the egg had degenerated. Counts were made of total and small ovarioles in a number of locusts in different stages. The number of individuals in each case is shown in brackets.

5th-instar nymph	Adults				
	Stage I	Stage II	Stage III	Stage IV (young)	Stage IV (old)
82 (4)	83 (15)	94 (12)	90 (14)	89·3 (17)	93·4 (22)

Young IV stage adults are less than two months old and old adults more than that age. There is thus no evidence here that the number of ovarioles decreases with age.
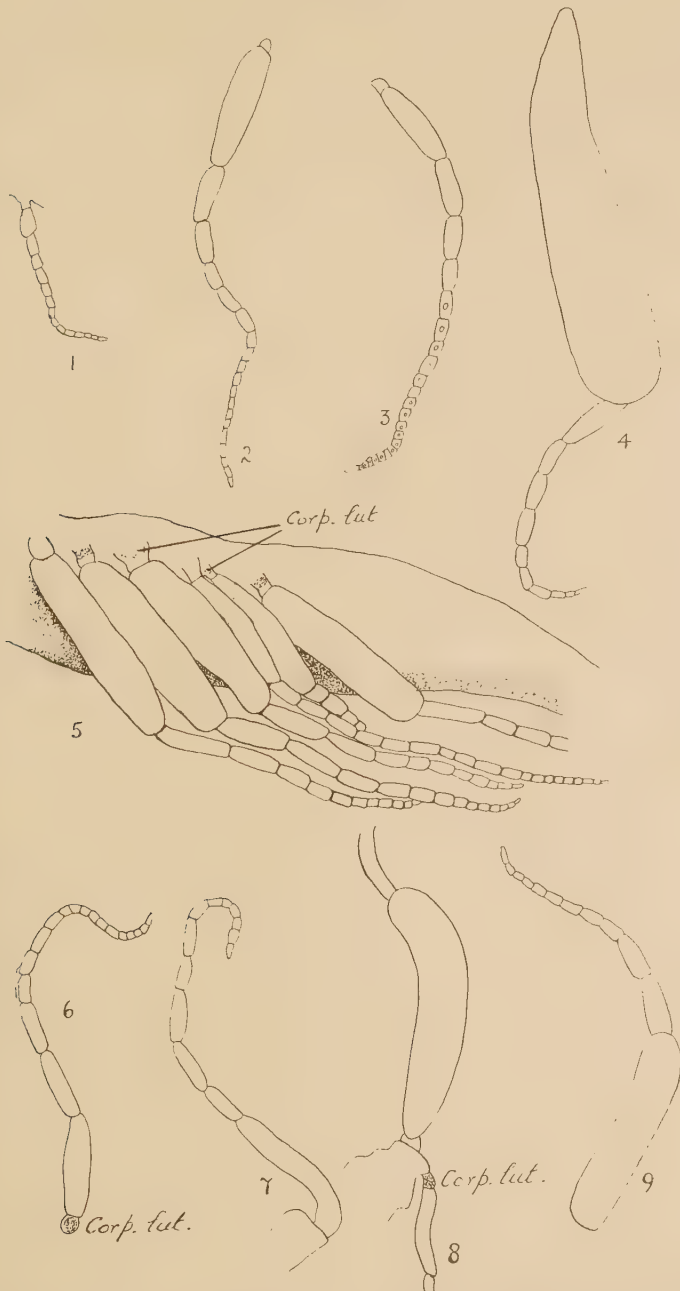
Figs. 1–9.—(1) Ovariole from Stage I ovary ; (2) Ovariole from late Stage II ovary ; (3) Ovariole from early Stage II ovary ; (4) Ovariole from Stage III ovary ; (5) Portion of Stage IV ovary showing *corpora lutea* and one small ovariole ; (6) Ovariole, with *corpus luteum* from Stage IV ovary of female with eggs in oviduct ; (7) Ovariole from Stage IV ovary of female with eggs in oviduct ; (8) Two ovarioles from Stage IV ovary, one small with *corpus luteum*, one normal without *corpus luteum* ; (9) Ovariole from Stage IV ovary. No eggs in oviduct.

As regards asymmetry, one of four fifth-instar nymphs showed no asymmetry and the other three differences of 1, 2 and 5 ovarioles between the two ovaries, the average difference being 2.

Stage	No. of individuals	Differences			
		None	0–2	Average	Max. diff.
I	15	2	Per cent. 66·7	2·1	5
II	12	3	66·7	1·7	5
III	14	2	43	4·2	5 (24)*
IV (young)	18	7	72·2	1·5	5
IV (old)	24	5	50·0	3·8	17

*One specimen showed 24, but this may well be abnormal, as the next highest was 5.

I am indebted to Mr. P. Robinson, Statistician, East African Agricultural and Agricultural and Forestry Research organisation, for the statistical treatment of these differences. They are arranged in a negative logarithmic distribution (neglecting the difference of 24 in stage III) and it seems probable that the asymmetry in stages II and III is not significantly different, but that significant differences exist between stages IV (young) and IV (old) and also between each of these and stages II and III. This increase in asymmetry with the onset of oviposition and also late in life would appear to require the loss of some ovarioles, a result which is at variance with the earlier conclusion based on the constancy of ovariole number. Further investigation of this point is required.

Turning now to the question of small ovarioles, the average number of these in four fifth-instar nymphs was 1·0 (1·2 per cent.), in 15 stage I adults nil, in 12 stage II adults 1·5 (1·6 per cent.), in 14 stage III adults 29·0 (32·4 per cent.), in 17 young stage IV adults 22·8 (25·5 per cent.), and in 22 old stage IV adults 40·8 (43·6 per cent.). It is clear that an increase in the number of small ovarioles occurs when the eggs are nearly ripe, and a further increase occurs in old adults. It is reasonable to suppose that small ovarioles are developed as a result of competition between the ovarioles for the available food, since they become numerous only when the demand for food increases. The second increase in numbers in old stage IV locusts probably indicates that the emergence of fewer nymphs from pods laid by old females is in fact due to the pods containing less eggs. It was thought that if the production of small ovarioles is due to competition for food, then the number of normal-size ovarioles within any stage should be less variable than the total number of ovarioles, since it should be controlled mainly by the food supply and not be affected by initial differences in number of ovarioles. The standard deviation of total ovarioles and normal ovarioles was, accordingly, calculated for each class. These standard deviations were in stage III, 15·5 and 13·0 ; in stage IV (young) 15·1 and 12·2 ; in stage IV (old) 16·3 and 23·2, all for total and normal ovarioles respectively. My thanks are again due to Mr. P. Robinson for the method of testing the significance of the differences between these standard deviations. The difference between total and normal ovarioles is significant in every case, indicating less variability in the number of normal ovarioles than in the number of total ovarioles in stage III and young stage IV, but more variability in normal than in total ovarioles in old stage IV. This latter difference must be connected with the second increase in small ovarioles.

It is also found that total ovarioles are significantly more variable in old stage IV than in either of the other two stages tested. This, like the increase in asymmetry, appears to require the loss of some ovarioles. Most of the old stage IV locusts dissected had spent their whole lives in the laboratory of the Anti-locust Research Centre, while most of the others were bred by the author, from stock originally obtained from the Centre. It is, therefore, not impossible that differences may have existed in the stocks or may have arisen as a result of feeding, which led to those from the Centre being more variable as to ovariole number and also having a greater tendency towards asymmetry. It appears highly desirable, in view of the conflicting evidence, to repeat these observations with larger numbers of locusts which have been reared together from the same stock.

Seven females were dissected with eggs in the oviducts. The average number of eggs extracted from each was 41·1. The total eggs in the oviduct was subtracted in each case from the number of normal ovarioles, the average of the differences being 23·0. Adding these two figures and also the average number of small ovarioles for young stage IV, i.e. 22·8, we obtain 86·9 which is in fair agreement with the average ovarioles for the stage, given above as 89·3. It thus appears that in addition to the small ovarioles an approximately equal number of normal ovarioles is non-functional. They are probably not permanently so, and it is quite possible that the ovarioles which have produced eggs become non-functional for a few days, while those previously non-functional become mature and ovulate.

The number of egg rudiments.

The number of rudiments per ovariole was estimated for each individual, in order to determine whether new egg rudiments are produced during adult life. All the rudiments in five ovarioles of one ovary, were counted and divided by five. It was found that different ovarioles of the same ovary had approximately the same number of rudiments, and it is believed that estimates obtained in this way are reliable.

Average values for rudiments per ovariole were next calculated for groups of individuals of the same stage and approximately the same age. They vary from 15·1 in 10 stage IV locusts between 20 and 50 days old to 17·6 in 6 stage II locusts 20–25 days old. None of the differences is significant. It may, therefore, be concluded that new rudiments are not produced in any numbers during stages I, II and III when no oviposition is occurring, but that they are produced in stage IV, and then at such a rate as to compensate for oviposition.

Further evidence supporting this conclusion is obtained from five females, which oviposited a known number of times. The sums of egg pods produced and number of rudiments per ovariole when dissected for these five were 19·6, 21·5, 22·0, 22·6, and 24·0. Only three females of all those dissected were found to have more than 20 rudiments per ovariole and the maximum ever found was 21, so this result is considered to yield evidence that new rudiments were produced during the adult life of these five individuals.

Rubtzov (1934) bases estimates of potential fertility of Siberian grasshoppers on counts of the number of rudiments per ovariole in newly emerged adults. This method does not appear to be applicable to *L. migratoria migratorioides*, under the conditions of the present experiments at least, and it would appear advisable to check whether rudiments are continuously produced in wild populations before any estimates of potential fertility are made on a basis of dissections of young adults.

Copulation.

Copulation and age.

Hamilton (1936) states that if immature females are put with males three to four weeks old, the time required to the first egg pod is reduced from about 14 days to about 13 days. In the present series of experiments some observations were made

on the time of occurrence of copulation, and it was noted that where males and females of the same age are kept together, the males attempt to copulate some days before the females are ready to permit it.   In one case, attempts on the part of the males to copulate were noticed when the oldest males in the cage were only four days old.   These attempts continued during the next five days, at the end of which two pairs were found to be copulating.   The two males were at this time 8 days and 9 days old respectively, and the two females 7 days and 8 days.   From this and other similar observations it is clear that the beginning of copulation is determined by the females, and the presence of older males will not ensure earlier copulation. It was also found that spermatozoa were present in the spermatheca of the youngest females copulating.

The influence of pairing on maturation.

It was stated by Pospelov (1934) that the ovaries of *Locusta* do not become mature if pairing is not permitted.   This conclusion was based on experiments carried out on only three female locusts, which were kept without males for four months.   Two were then dissected and found to be immature.   The remaining one was allowed to pair and laid eggs seven days later.

Hamilton (1936) found that unmated *Locusta* became mature, but at a slower rate than mated ones, and oviposited.   Two series of experiments were undertaken, one during March and one in April to investigate this point.

March series.

Two female locusts which became adult on the same day were kept, one with females only throughout life, while the other was put with males at the age of 5 days, and returned to the cage with females only at the age of 12 days.   Both locusts were dissected at the age of 24 days, when the female which had paired was found to be in stage IV, while the one which had not was in stage I.

In another case four females, all 22 days old, two which had not been allowed to pair were in stage II while the other two, which had paired were in stage III and stage IV respectively.

During the whole March series no unmated female ever became mature, though many were kept for over three weeks and during the same period mated females regularly became mature and oviposited.

Throughout these experiments the grass supplied to the locusts was rather small in quantity and certainly poor in quality, owing to the severe weather.

April series.

Some locusts were kept in cages under conditions similar to those obtaining in the March series, while others were kept in the 7-lb. glass jars mentioned above, 4 in each jar.   Two of the jars contained four females each, th other six jars each contained 2 males and 2 females.   The females were weighed regularly and the jars were examined every day for egg pods.   The locusts kept in cages were also weighed, but the number of pods laid was not recorded.

In both jars and cages, the unmated females became mature and oviposited. The 12 mated females laid an average of 2·6 pods each and the mean age when the first pod was laid was 16·4 days.   The 8 unmated females laid an average of one pod each and the mean age when the first pod was laid was 18·3 days.   Thus, the average age at first oviposition was higher and the number of pods per female less in unmated than in mated females.
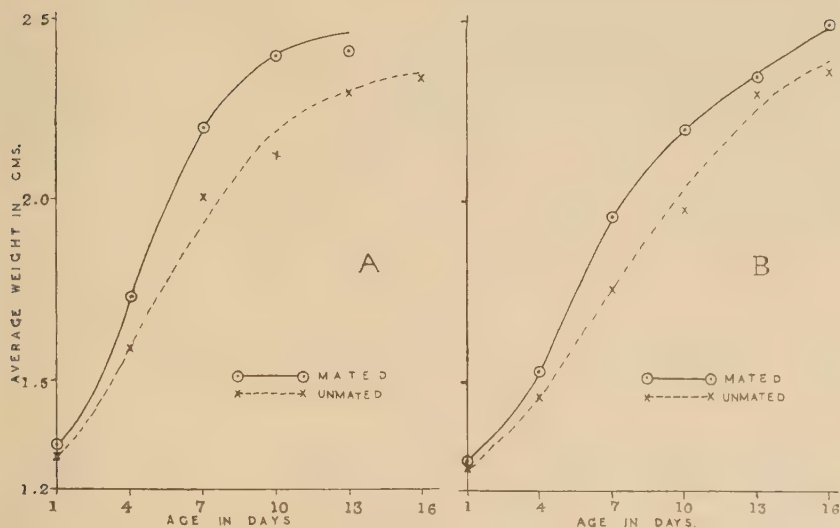
Fig. 10.—Growth curves for : (A) Mated and unmated locusts in cages ; (B) mated and unmated locusts in jars.

The results of weighing two lots of mated and two of unmated females also indicate that the rate of increase of weight is less rapid in unmated than in mated females kept under the same conditions. The curves shown in Fig. 10 (A) were obtained from the weights of two sets of locusts kept in the cages and those in Fig. 10 (B) from two other sets kept in the jars. It is shown later that a relation exists between weight and maturity, and that a less rapid rate of weight increase indicates a postponement of maturity.

The locusts were supplied with plenty of grass of good quality during the April series of experiments. It is considered that the results justify the conclusion that copulation stimulates the development of the ovaries, as suggested by Hamilton (1936), and suggest that this stimulus is essential to maturation if the food supply falls below a certain level. With abundant good food and otherwise favourable conditions the ovaries mature without copulation, but they do so rather more slowly and fecundity is reduced. It is possible that a similar relation exists between other factors such as humidity and temperature, and that in Pospelov's (1934) experiments some factor unfavourable to the attainment of maturity was present, as in the experiments of the March series described above. It is interesting to note that Hamilton (1936) found that eggs laid by unfertilised females did not hatch. In the present investigation some of the unfertilised eggs hatched, but the hoppers were too few to be successfully reared and died in the first instar. The sex was not determined.

Weight and Maturity.

Weight and stage.

Table I exhibits the average weight of locusts in each of the four stages into which the process of maturation has been divided. The number of locusts and the standard deviation are given in each stage. The figures are based only on locusts dissected, of which the stage is accurately known by observation, and each stage includes locusts of different ages. In stage I are included locusts between one and

18 days old, the average age being 12·3 days. In stage II are included locusts between 9 and 25 days old, the average age being 14·4 days, while stage III includes locusts between 11 and 25 days, the average age being 16·3 days. These figures are only mentioned here in order to show that the average weights for stage I are not calculated mainly on very young locusts and those for later stages on much older individuals. The relation between weight and age is dealt with more fully in a later section.

<div align="center">TABLE I.</div>

	No. of locusts	Average weight (gm.)	σ
Stage I	40	1·837	0·40
Stage II	24	1·966	0·249
Stage III	24	2·486	0·377
Stage IV	45	2·310	0·30

Tests of significance show that the difference between stages I and II and stages III and IV are not significant, while the differences between stages I and III, II and III, I and IV and II and IV are highly significant. In general, it may be stated that the average weight of a population of locusts which is ovipositing or is ready to oviposit, is significantly greater than the average weight of a population which is not sexually mature.

As a further check on this conclusion, use was made of 23 locusts between 13 and 18 days old, which were still in stage I. Their failure to mature was due to the rather low temperatures, between 15°C. and 20°C., at which they had been kept, but they had been regularly fed and had increased in weight. The average weight of these locusts was compared with that of all stage III locusts not more than 18 days old. The average weight of the 23 stage I locusts was 2·03 gm. (σ=0·294) and of 18 stage III locusts of the same age was 2·40 (σ=0·525). The difference between the two averages is significant, corresponding to P<0·01, thus affording further evidence that weight depends primarily on the development of the ovaries.

An attempt was first made to correlate stages in maturation with the ratio weight/wing length, in order to introduce a correction for initial differences in size. It was later found that the use of weight alone gave the same results as the use of the ratio, and that there was no significant difference between the coefficient of variation of weight and that of the ratio. The labour of calculating the ratio was therefore dispensed with.

Weight and age.

There is certainly a relation between the weight and the age of a locust. This is indicated above, where the average weight of a number of stage I locusts over 13 days old is higher than that obtained by including all stage I locusts, irrespective of age.

Fig. 11 shows the increase of weight with age. It is based on figures obtained in three different experiments, in two of which locusts were weighed at regular intervals throughout life. In the third experiment weighing was carried out less regularly. The locusts were kept at temperatures ranging from 30° to 37°C. and relative humidities ranging from 40 to 80 per cent., so the curve indicates the average weight at any age of a mixed population. The minimum number of weight readings used in calculating any one point on the curve up to the age of 18 days was 25, the maximum was 94. Most of the points are calculated from between 30 and 50 individual weights.

The curve indicates that the rate of increase in weight is rapid at first, then reduced for about two days and afterwards increased to about its initial value. This rate is maintained until the weight is about 2 gm., when it is again reduced. At about 14–15 days oviposition commences, after which the weight fluctuates rather irregularly. Probably this part of the curve can be represented by a straight line, mainly horizontal. A study of individual weight records indicates that the weight of a female frequently rises to a higher value between successive ovipositions than any value which was attained before the first oviposition, and that there is a rather sudden fall just before death, so a steady fall in weight during stage IV would probably not occur, even if the averages were calculated from some hundreds of individual weights.
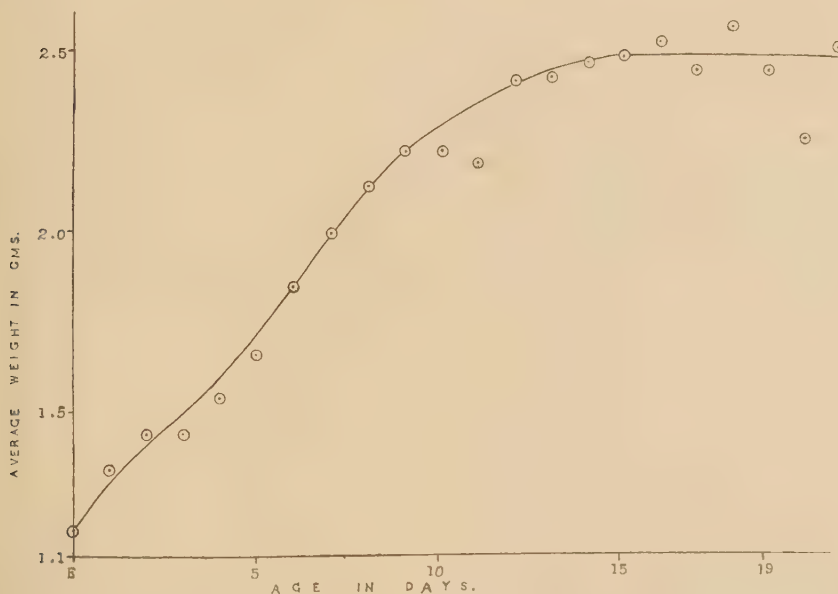
Fig. 11.—Growth curve for a population of mated female locusts.

The reduction in the rate of growth between the ages of 2 and 4 days is possibly not significant. It is due largely to the occurrence of a low weight value at three days in one of the experiments, presumably because of temporarily unfavourable conditions, but the other experiment from which continuous records are available also shows a slight fall in the rate of growth between the third and fourth days, and some indication of the same phenomenon is seen in the graphs of fig. 10. It is therefore possible that a tendency exists for rather less rapid growth to occur at about 3–5 days. This is discussed below.

The relation between weight and age is further complicated by the relation between weight and maturation and the dependence of maturation on temperature, humidity and food supply. These factors may influence weight and maturation independently, or they may exert their effect on weight only through their effect on maturation. It appears to be impossible to design an experiment to show which of these two alternatives is correct, but the difference is unimportant since the effect is the same in both cases. The effect of unfavourable conditions in retarding increase in weight and maturation is illustrated in fig. 12. This curve is not based on regular weighings of locusts, but on the weights of locusts of known age when dissected. The locusts

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were first divided into stages I, II, III and IV, and each stage was then subdivided according to age. Locusts 1–3 days old were included in the first group, 4–6 days in the second, and so on. This grouping was necessary in order to obtain a sufficient number of weight readings in each group.
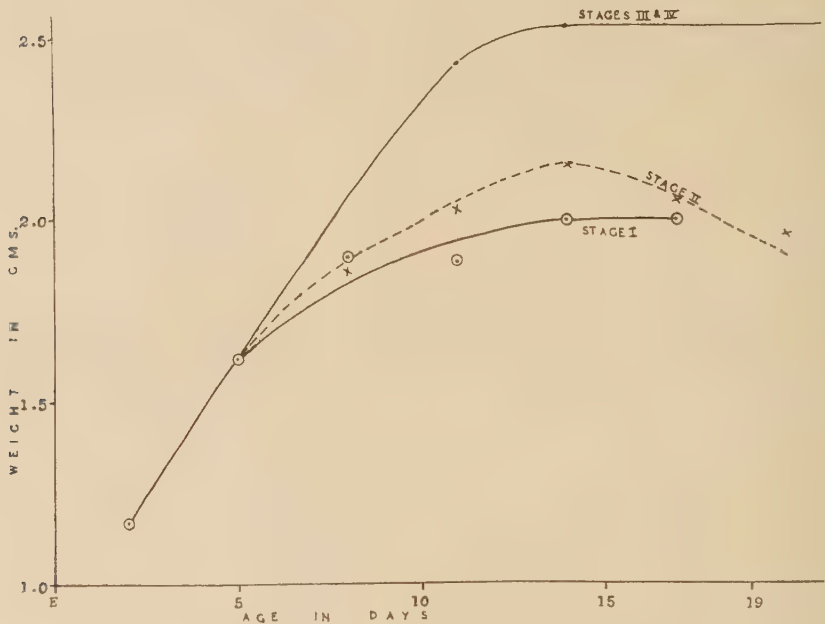
Fig. 12.—Weight increases of female locusts in different stages.

Amongst the locusts shown as remaining in stages I and II are those referred to as old stage I in the section on weight and stage. These, which did not mature, because of unfavourable conditions, increased to a certain weight, after which the weight remained constant, or fell. The fall was well marked in stage II locusts over 14 days old, and would probably have occurred also with stage I if they had been kept longer.

Where the ovaries become mature, the weight rises rapidly, and the curve for locusts proceeding to stage III diverges from that for those remaining in stages I and II at about 5–6 days. Actually, no stage III locusts less than 10 days old were dissected, so the form of the part of the curve before this is uncertain. However, if it is made to diverge much later than 5 days, a pronounced S-shape is obtained. The possibility of the occurrence of such a shape, at a rather earlier age, has been mentioned above, and it now seems likely that the kink, if it occurs, indicates some change connected with further growth of the eggs, possibly the beginning of the transfer of fat to them from the fat body. Further discussion of this point is not likely to be profitable until more evidence is available.

Some records were kept of the weights of males, and from these the curve shown in fig. 13 was drawn. The rate of increase of weight is at no time as rapid as in the females, but it attains its greatest value between 5 and 8 days, as in the females. It flattens out at about 10 days, while the females continue to increase in weight up to 14–15 days. After attaining the maximum value the weight fluctuates irregularly.

These fluctuations, expressed as a percentage of the mean weight, are as large as the fluctuations in the weight of stage IV females expressed in the same way.
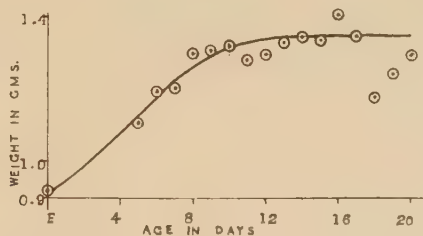
Fig. 13.—Growth curve for a population of male locusts.

Weight and Egg-size.

The same set of results as for weight and age was used to investigate the relation between weight and egg-size. As before, the locusts were divided into stages, and then each stage was subdivided according to the length of egg_1, measured with an eyepiece micrometer. The results for stages III and IV are summarised in fig. 14. There is little relation between weight and egg-size in stages I and II. This result might have been expected, since during this period the fat body is growing rapidly, and much of the increase in weight is due to this.
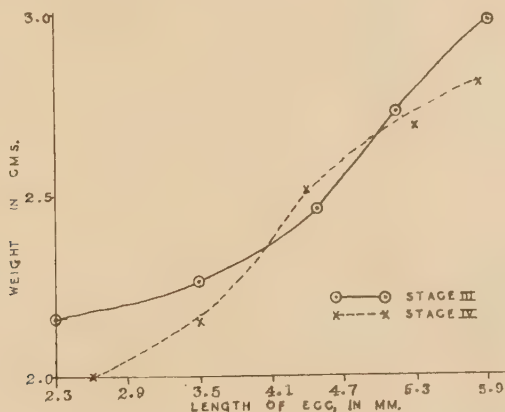
Fig. 14.—The relation between weight and egg-size in female locusts.

In stages III and IV the relation is closer. The rate of increase of weight with increase of egg-size is at first low, and probably during this period much of the increase in egg-size is due to the transference of fat from the fat body to the eggs. Above an egg length of about 4·5 mm. the rate of increase of weight becomes much greater and this is presumably due to the laying down in the eggs of substances derived directly from the food.

The first stage IV individuals are lighter and have smaller eggs than any of those in stage III, but the curve has the same form as that for stage III. The highest average weight observed in stage IV was lower than that in stage III, but all of these averages were calculated from specimens which had been dissected, and rather few weight readings were available for calculation in some groups.

The average weight of 13 locusts with eggs in the oviduct was 2·438 gm. This value is lower than that for locusts with egg_1 5·8 mm. long, but this result is to be expected, since the size of mature eggs varies rather widely and those with eggs in the oviduct will include many in which mature eggs were small. These 13 locusts were divided into groups according to the length of the eggs in the oviduct, and the average weight calculated for each group. The results indicate that there is a tendency for heavier locusts to lay larger eggs. No relation was found between egg size and femur length.

Growth of Eggs.

It is now possible to use the results shown in figs. 11 and 14 in order to obtain some idea of the rate of growth of the eggs with respect to age. It will be noted that average weights above 2·6 gm. were not obtained in figs. 11 and 12, but were obtained in fig. 14. This is, of course, because in separating the locusts according to egg-size, the heaviest of the mature females are grouped together, whereas in separating them according to age, even if they are all mature, some with smaller eggs will be included and will give a lower average.
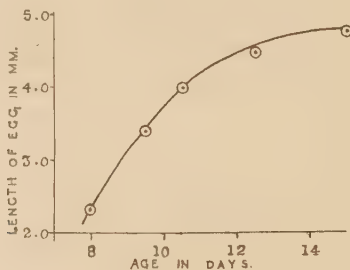
Fig. 15.—Rate of growth of egg_1.

Combination of the results as far as is possible gives the curve shown in fig. 15. This applies only to stage III locusts, since it has been shown that there is little relation between weight and egg-size in stages I and II. The rate of growth of the eggs is rapid at first, but slows down at about 12 days, when the eggs are almost mature.

It has been mentioned that the size of mature eggs varies rather widely between individuals. Eggs were removed from the oviducts of 13 females. The smallest were 6·5 mm. long and the largest 8·0 mm., the average length being 7·0 mm. In view of the small size of egg_1 in those individuals with eggs in the oviduct, an attempt was made to determine whether ovulation is followed by rapid growth of egg_1. The stage IV females were divided into two groups, those with eggs in the oviduct (recently ovulated) in which the average length of egg_1 was 1·9 mm. and those without eggs in the oviduct (not recently ovulated) in which the average length of egg_1 was 3·5 mm.

Thus the average size of egg_1 in females that had not recently ovulated is nearer to the size in recently ovulated females than to the average size of an egg from the oviduct. It is considered that this result indicates that ovulation is followed by a period of rather slow growth of egg_1. This phase cannot last very long, since it was frequently noted that not more than one day elapsed between successive ovipositions.

Water and Fat Content of Locusts.

Some estimates were made of the water and fat contents of locusts of different ages, with the object of discovering what is responsible for the increase in weight observed during maturation. Locusts of known age were weighed, killed and dried in tins over silica gel at about 80°C. for about 12 hours. Further drying after this time resulted in very little more loss of weight, and it was assumed that all the water had been extracted. They were then stored in corked tubes with a small quantity of silica gel to keep them dry until the fat extraction was carried out some months later.

The fat was extracted in a Soxhlet apparatus, using diethyl ether as solvent. No separation of the extract was made. For purposes of fat extraction, a number of locusts of about the same age and water content were ground up together, consequently each value of percentage fat represents an average of a number of individuals.

The results are shown in Table II. Ages are given in days, E indicating newly emerged adults. Water and fat contents are given as percentages of the live weight, and the number of individuals used in each determination is also given.

TABLE II.

Age in days	No. of locusts	Total live weight gms.	Estimated stage	Per cent. water	Per cent. fat	Per cent. water + per cent. fat
E	4	4·781	I	72	6	78
2	7	7·689	I	74	6	80
7–8	2	4·933	III	60	12	72
11–12	3	6·208	III	59	13	71
14–15	2	5·326	III	56	15	71
16	3	8·034	IV	62	10	72
20	2	5·501	IV	58	14	72
18–24	3	8·393	IV	65	9	74
26–27	2	5·807	IV	61	8	69
29–32	6	13·544	IV	64	8	72
38–39	2	4·134	IV	65	8	73

The water content expressed as a percentage of the live weight thus falls rather rapidly during the first week, after which it remains constant. The differences between percentage water at different ages after 7 days cannot be regarded as significant, as they are no greater than the differences found between individuals of the same age. For this reason percentages are given only to the nearest whole number.

The fat content shows a fairly rapid rise up to the time when oviposition commences and the continuation of this rise until the eggs are ripe, and during the period when the fat body is decreasing, supports the earlier conclusion that during the later stages of egg growth fat derived directly from the food is used. The percentage fat falls as oviposition proceeds, but it is not significantly lower in locusts 40 days old than in those 24 days old. In this connection it may be remembered that the weight of stage IV locusts shows no steady fall during the oviposition period.

The occurrence of a high water content and low fat content at 16 days is quite inconsistent with the other observations. The three locusts were probably stage IV, and it may be that one or more of them had just oviposited, so considerably reducing its fat content. It is difficult to see why the percentage water should rise simultaneously, but it was noted that the sum of per cent. water and per cent. fat is approximately constant after the age of 7 days. There was unfortunately insufficient

time for experiments to show whether this constancy would always be found. If it is, it may indicate that after oviposition feeding and digestion are rapid, resulting in a large water intake, and that the food is then converted into fat for the developing eggs, while excess water is excreted.

If the percentages of water and fat are now expressed as actual weights of water and fat in an average locust which grows and matures as in fig. 11, it is found that the weight of water in the body increases during growth. The results are shown in Table III, up to the age of 15 days, after which weight and percentage water are assumed constant.

TABLE III.

Age	Water content in gms.	Fat content in gm.	Total live wt. gm.
E	0·864	0·072	1·2
2	1·036	0·084	1·4
7–8	1·230	0·246	2·05
11–12	1·392	0·312	2·4
14–15	1·400	0·375	2·5

Almost half of the weight increase is therefore due to water intake.

Bodine (1921) made estimates of the water content of various stages of *Melanoplus femur rubrum*, *Dichromorpha viridis* and *M. differentialis*. He showed that percentage water decreases with increasing weight of the insects, not only in adults but also in nymphs. He does not test the significance of the differences noted, but an inspection of his Table I suggests that in these species too, an initial fall in percentage water is followed by a period in which no further significant change occurs. The percentage water is shown to be related to age rather than to body weight in *M. differentialis*, but the relation to weight clearly exists in the other species, and suggests that in non-swarming grasshoppers, too, a rise in weight and decrease in percentage water accompanies maturation.

Duarte (1938) also investigated the dry weight of female *L. migratoria migratorioides* and found 70·13 per cent. water in the newly-emerged females bred under crowded conditions.

Discussion.

The foregoing results apply, of course, only to locusts reared under certain experimental conditions. It has not been possible to find any record of the weight of wild locusts, nor even of locusts reared in outdoor cages. It is therefore uncertain to what extent the weights obtained are comparable with those of wild populations. It appears probable that, under any conditions, increase in weight would accompany maturation, but with a less homogeneous population some advantage might be obtained by using a ratio such as weight/elytron length or weight/femur length. With a sufficiently homogeneous wild population, weight alone would presumably be a good criterion of sexual maturity, and since an average only would be required, one pound of locusts could be weighed on a spring balance and the number afterwards counted. If the average weight is 2 gm., a little more than that found for stage II in the present investigation, about 230 locusts will be required for one pound. If the average weight is 2·5 gm., about that found for stage III, 182 locusts will weigh one pound.

The estimation of maturity from weight may be of some use in laboratory studies where homogeneous populations are used and where it is desired to obtain the stage of development without dissection. An example of the use of this method is therefore

given. Twenty locusts were used, which had emerged between 12th and 20th August, so that the maximum difference in age was eight days. The average weights of this population were on 12th August 1·234 ; on the 13th, 1·216 ; on the 14th, 1·280 ; on the 15th, 1·246 ; on the 16th, 1·280 ; on the 17th, 1·343 ; on the 20th, 1·345 ; on the 23rd, 1·742 ; on the 25th, 2·128, and on the 28th, 2·514 gms. It could fairly be concluded from these figures that maturity was attained by the population as a whole between 25th and 28th August, and that before 25th August very few of the locusts were ready to oviposit.

It may be objected that the weight depends on temperature and humidity, and that different results would be obtained if the locusts were reared under different conditions. It has already been shown that unsuitable conditions prevent maturation and at the same time prevent increase in weight above a certain value, so that weight and maturity go hand-in-hand either because weight depends directly on maturity or because the two qualities are simultaneously affected. As regards the possibility that variation in conditions during nymphal life may produce wide differences in weight, the results of rearing three sets of locusts may be considered. These three sets were reared at different times of the year, so that the quality of the grass supplied was not constant. The first two were reared chiefly in the glass tank, where the humidity was usually 80 per cent. and often rose to 100 per cent. after the provision of fresh grass, which was given several times a day. The third set was reared in one of the large cases of expanded rubber, where the humidity was seldom above 60 per cent. The degree of crowding was different in the three sets, being highest in the second. Weights at emergence are shown in Table IV.

TABLE IV.

	No. of locusts	Average weight at emergence in gm.	Max. weight	Min. weight
1st set ...	44	1·160	1·383	0·902
2nd set ...	50	1·217	1·503	0·968
3rd set ...	94	1·324	1·670	0·865

The total reared is 188 and the average weight at emergence 1·257 gm.

In the third set the variation is greater and the average weight higher, but the differences between the three averages are considerably smaller than those between individuals of one set. It may therefore be concluded that within the conditions of the experiment variations in temperature and humidity do not have any effect on weight without at the same time affecting maturation.

Duarte (1938) gives the average weight of newly-emerged female *L. migratoria migratorioides* bred under crowded conditions as 1·250 gm., but he apparently obtained a very much smaller standard deviation than that found in the present investigation. It appears, then, that the locusts reared in the experiments described above are comparable with other locusts reared by other investigators. How far they, and the results obtained, are comparable with wild populations, remains to be discovered.

Summary.

The objects of the investigation were to describe the process of maturation of the ovaries, to clear up the question of the relation between copulation and sexual maturity, and to determine whether any relation exists between weight and sexual maturity.

The locusts used were of the *transiens* phase. They were kept at temperatures between 32°C. and 37°C. and relative humidities between 50 per cent. and 70 per cent.

The maturation of the ovaries was followed, and is divided into four stages· distinguished chiefly by the sizes of the eggs.

It is shown that the average number of ovarioles per female does not change significantly with age.

The numbers of ovarioles in the two ovaries of an individual often shows asymmetry, which probably increases with age.

Some of the ovarioles are small. The percentage small ovarioles increases with development of the eggs. Variation between individuals in the total number of ovarioles also increases with age.

The number of egg rudiments per ovariole was calculated for different ages and stages. The differences were not significant. It is concluded that new rudiments are produced during the oviposition period.

It was found that when the food supply was relatively short, female locusts became mature and oviposited if allowed to pair, but did not mature if males were not present. With abundant food, unmated females matured, but more slowly than mated ones and fecundity was reduced. It is concluded that under unfavourable conditions pairing may be necessary for maturation of the ovaries.

Average weights of female locusts in each of the four stages were compared. Locusts in stages III and IV, i.e., those which are mature, were found to be significantly heavier than those in stages I and II. The average weight of a number of stage I locusts 13–18 days old (whose development had been retarded by unsuitable conditions) was also found to be significantly less than that of a number of stage III locusts of the same age. It is concluded that weight depends primarily on the development of the ovaries.

The relation between weight and age was investigated. Rate of increase of weight is rapid at first, is reduced for about two days, then increases again and maintains its value until the weight is about 2 gms. During the oviposition period it fluctuates irregularly.

It is shown that if conditions are unfavourable to maturation the weight rises to a certain value, after which it remains constant, or falls. The fall was best seen in stage II locusts over 14 days old.

There is little relation between weight and egg-size in stages I and II, but in stages III and IV the relation is closer. Rate of increase of weight with egg-size is at first low, but above an egg-length of about 4·5 mm. the rate of increase of weight becomes greater. It is suggested that this is because early growth of the eggs is due to transference of fat from the fat body and later stages to the laying down in the eggs of substances derived directly from the food.

Estimates were made of water and fat content of locusts of various ages. Percentage water falls rather rapidly in the first few days of adult life after which it remains approximately constant. Percentage fat rises to a maximum at about 14 days, then falls slowly. Most of the increase in weight during maturation is due to water intake.

Acknowledgements.

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GENERAL INDEX.

carnea, Chrysopa.

carnifex, Scolia.

Carum copticum, **301.**

Cassava, *Laemophloeus ferrugineus* infesting, **64.**

castaneum, Tribolium.

Catabomba pyrastri, effect of DDT and BHC on, **281–287.**

Cattle, control of *Boophilus decoloratus* on, with " Gammexane " in S. Africa, **207–226 ;** as bait for *Glossina*, **44** ; control of *Glossina* with DDT-treated, in Tanganyika, **123–134 ;** and trypanosomiasis in Kenya, **525–527.**

Cecidomyiids, predacious habits of, **119** ; effects of insecticides on, **487, 489.**

Ceiba pentandra, **149.**

cembrae, Cinara (Lachnus).

centurionis, Chrysops.

cephalonica, Corcyra.

cerealella, Sitotroga.

Chalk, and mercury against pests of stored grain, **301.**

Charips spp., parasites of *Aphidius*, **98, 106.**

Cherry, *Quettania coeruleipennis* on, in Quetta, **203.**

Chillies, *Laemophloeus* infesting, **64.**

chloropyga, Chrysomyia.

Chrysanthemum, systemic insecticide against Aphids and Coccid on, **483.**

chrysogaster, Eretmapodites.

Chrysomyia spp., pupation habits of, affecting parasitism, **533–537.**

Chrysopa carnea, predacious on Aphids, **118.**

Chrysops centurionis, in Uganda, **170.**

Cimex lectularius, insecticides against, **135.**

Cinara (Lachnus) cembrae, Platychirus peltatus predacious on, in Austria, **108.**

Cineraria, systemic insecticide against Aphids on, **483.**

circumflexum, Aulacorthum.

circumluteolus, Aëdes (Banksinella).

citri, Pseudococcus.

clavata, Coruna.

coarctata, Leptohylemyia.

Coccinella septempunctata, characters and bionomics of, **113** ; effect of DDT and BHC on, **287–291.**

Coccinellids, effects of insecticides on, **486, 489.**

Cockroaches, effect of systemic insecticide on, **486.**

Coconut, *Oryctes rhinoceros* on, in Samoa, **445.**

coeruleipennis, Quettania.

Coffee, *Laemophloeus minutus* infesting, **63 ;** experiments with, for loss of insecticides by absorption, **334.**

Coleus, systemic insecticide against Coccid on, **483.**

confusum, Tribolium.

Copper Oxychloride, in spray against cotton Jassid in Sudan, **85, 92.**

Copra, *Laemophloeus* infesting, **63, 64.**

Coquillettidia (see *Taeniorhynchus*).

Corcyra cephalonica, tests with mercury against, **301–303.**

Cordia macrostachya, introduction of *Physonota alutacea* into Mauritius against, **479–480.**

cornutus, Gnathocerus.

corollae, Syrphus.

corporis, Pediculus humanus.

Coruna clavata, parasite of *Aphidius*, **105.**

Cotton, control of *Empoasca lybica* on, in Sudan, **83–96 ;** systemic insecticide against Jassid on, **483.**

Cottonseed, *Laemophloeus minutus* infesting, **63.**

Cottonseed Oil, as solvent for DDT, **326.**

coustani, Anopheles (A.).

Cowries (see *Cypraea*).

crataegarius, Ovatus.

Cryptomyzus ribis, **116.**

Culex spp., in trees in Uganda, **170–173.**

Culex andreanus, in S. Nigeria, **387.**

Culex decens, in S. Nigeria, **387.**

Culex guiarti, in S. Nigeria, **387.**

Culex inconspicuosus, in S. Nigeria, **387.**

Culex ingrami, in S. Nigeria, **387.**

Culex insignis, in S. Nigeria, **387.**

Culex perfidiosus, in S. Nigeria, **387.**

Culex perfuscus, in S. Nigeria, **387.**

Culex philipi, in S. Nigeria, **387.**

Culex poicilipes, in S. Nigeria, **387.**

Culex rima, in S. Nigeria, **387.**

Culex subrima, in S. Nigeria, **387.**

Culex sunyaniensis, in S. Nigeria, **387.**

Culex thalassius, bionomics of, in S. Nigeria, **387–402.**

Culex tigripes, in Uganda, **173.**

Culex wigglesworthi, in S. Nigeria, **387.**

cumminsi, Aëdes (Aëdimorphus).

curvicornis, Charips.

cyaneus, Sirex.

Cyclohexylamine Dodecyl Sulphate, **6, 239, 252.**

Cynometra, **149.**

cyparissae, Macrosiphum.

Cypraea, **203.**

D.

D.P. 530, **332.**

dalmani, Aegilips.

DDT, experiments with, against insects and ticks, **6–24, 85–95, 123–134, 135–148, 211, 239–265, 279–297, 323–343, 357, 379–385, 403–429, 431–444.**

de-boeri, Aëdes (Stegomyia).

decens, Culex (C.).

decoloratus, Boophilus.

de-meilloni, Aëdes (Stegomyia) de-boeri.

Dermestes vulpinus, oviposition cycle of, **69.**

Derris, effect of spray of, on aphidophagous insects and their hosts, **285–291.**

destructor, Tribolium.

detorquens, Technomyrmex.

detritum, Hyalomma.

Diaeretus rapae, parasite of Aphids in U.S.A. and Hawaii, **98.**

Diceromyia (see *Aëdes*).

Dichromorpha viridis, water content of, **554.**

Diesoline, as solvent for DDT, **326.**

differentialis, Melanoplus.

bis (bis Dimethylamino phosphonous) Anhydride, properties and use of, as a systemic insecticide, **481–501.**

Dinitro-o-cresol, effect of humidity on toxicity of, **439.**

Ditrene, **331.**

DNC (see Dinitro-o-cresol).

Dog, toxicity of systemic insecticide to, **493.**

domestica, Musca.

domesticus, Aëdes (Aëdimorphus).

dominica, Rhizopertha.

Doralis (see *Aphis*).

Drepanosiphum platanoides, **116.**

Drosicha mangiferae (octocaudata), food-plants and morphology of, in India, **351–353 ;** probably a synonym of *D. stebbingi,* **353.**

Drosicha stebbingi, food-plants and morphology of, in India, **351–353.**

Dunnius (see *Aëdes*).

E.

Empoasca lybica, control of, on cotton in Sudan, **83–96 ;** systemic insecticide against, on cotton, **483.**

Ephedrus spp., parasites of *Myzus persicae* in Canada and U.S.A., **98.**

Ephestia kühniella, tests with, on persistent toxicity of insecticides, **135–148.**

Eretmapodites spp., in trees in Uganda, **172–173.**

Eriosoma lanigerum, systemic insecticide against, on apple, **483.**

Eristalis spp., effect of DDT against Aphids on, **283.**

ervi, Aphidius.

Ethiopia, bionomics of *Phymateus pulcherrimus* in, **362.**

Euphorbia, Phymateus aegrotus destroying, in E. Africa, **360.**

euphorbiae, Macrosiphum.

Eutettix tenellus, effect of humidity on toxicity of insecticides to, **443.**

evertsi, Rhipicephalus.

F.

fabae, Aphis (Doralis).

farauti, Anopheles (Myzomyia).

Farex, mosquito larvae bred on, **32.**

fasciata, Phalacrotophora.

femur-rubrum, Melanoplus.

ferrugineus, Laemophloeus.

Ficalbia spp., in S. Nigeria, **387.**

Finlaya (see *Aëdes*).

Fish River Bush Tick (see *Haemaphysalis silacea*).

flavicollis, Aëdes (Diceromyia).

floricola, Monomorium.

Flour, *Laemophloeus* infesting, **63, 64.**

fragariae, Capitophorus.

funestus, Anopheles (Myzomyia).

Fungi, destroying Aphids, **120.**

funicola, Siphunculina.

fuscopennatus, Taeniorhynchus (Coquillettidia).

fuscum, Tetropium.

G.

gabrieli, Tetropium.

gambiae, Anopheles (Myzomyia).

" Gammexane," control of *Boophilus decoloratus* with, in S. Africa, **207–226 ;** against cotton Jassid in Sudan, **86–87 ;** persistent toxicity of, **135–148.**

geminata, Solenopsis.

Glossina, effect of climate on activity of, in Kenya, **307–321.**

Glossina austeni, bionomics and control of, in Kenya, **43, 307–321, 345–349, 512, 513.**

Glossina brevipalpis, bionomics and control of, in Kenya, **43, 307–321, 345–349, 511–531.**

Glossina longipennis, carried by railway trains in Kenya, **511–531.**

N.

O.

INDEX TO NAMES OF PERSONS.

Adam, N. K., **509.**
Ahmad, Manzoor, **303.**
Anscombe, F. J., **377.**
Ashby, D. G., **481.**
Aspey, J. A., **377.**

Barker, C. H., **481.**
Barlow, F., **323.**
Bates, R., **500.**
Bathgate, J., **292, 295.**
Bax, S. Napier, **133.**
Bedford, H. W., **96.**
Benson, H. J. Craufurd-, **428.**
Berger, N. E., **355.**
Bharihoki, —, **303.**
Bianchi, F. A., **445.**
Boyd, D. A., **500.**
Bradford, B., **207.**
Brett, G. A., **135.**
Bugher, J. C., **168.**
Bunzli, G. H., **481.**
Buxton, P. A., **509.**

Cooper, B. A., **277.**
Cooper, F. A., **428.**
Cowland, J. W., **83, 481.**
Craufurd-Benson, H. J., **428.**

Davies, G., **478.**
Davies, M., **428.**
Davies, R. G., **63.**
de Wilde, F., **226.**
Dold, D. A. L., **226.**
Dumbleton, L. J., **446.**
Dunn, J. A., **97.**

Eames, —, **233.**
Edwards, C. J., **83.**
Emden, F. van, **61.**
Evans, G. O., **459.**

Fairley, N. H., **41.**
Feldman-Muhsam, B., **305.**
Finney, D. J., **186, 201.**
Fitzherbert, P., **41.**
Ford, D., **226.**
Ford, F. P., **226.**
Ford, G. V., **226.**
Ford, H. J., **226.**
Freeman, P., **454.**

Gaitskill, A., **96.**
Gardner, J. C. M., **206.**
Gibson, N. H. E., **277.**
Gillham, Mrs. E. M., **24, 264, 444.**
Glasgow, J. P., **133.**
Goscombe, E. G., **481.**
Gough, H. C., **267.**
Gray, R. A. H., **121.**
Greenslade, R. M., **481.**

Hadaway, A. B., **323.**
Haddow, A. J., **169.**
Hall, T. D., **225.**
Hartley, G. S., **481.**
Heath, D. F., **481.**
Heatherington, W., **481.**
Herford, G. V. B., **80.**
Hertig, M., **453.**
Hopkins, B., **24, 264, 444.**
Howe, R. W., **80.**
Hughes, L. E., **478.**
Hull, R., **500.**
Hynes, H. B. N., **362–364, 368.**

Jackson, C. H. N., **47, 133, 320.**
James, L. E., **96.**
Janjua, N. A., **203, 303.**
Johnston, A. N., **447.**
Jones, B. M., **135.**
Jones, G. D. G., **277.**
Jones, T. W. Tyssul, **379.**

Kettle, D. S., **403.**
Kevan, D. K. McE., **359.**
Khan, A. W., **61.**
Krijgsman, B. J., **355.**

Latif, A., **351.**
Lawson, J. W. H., **428.**
Lawton, R., **277.**
Legg, A. W., **226.**
Lemerle, T. H., **27.**
Lewis, E. A., **511.**
Lickerish, L. A., **481.**

McArthur, J., **49, 53.**
McGillivray, I., **226.**
Mackerras, I. M., **41, 452.**
Mackerras, M. J., **27.**
MacLeod, J., **179.**